

LIVER    FUNCTION    TESTING.

by

ERNEST    BULMER

M.B., Ch.B., M.R.C.P.E.

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A SUMMARY OF THE LIVER FUNCTIONS

AND

THE METHODS OF TESTING THEM.

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The investigation of the functional capacity of the liver has long exercised the ingenuity of the medical world, and as knowledge of liver physiology has advanced so have the liver function tests increased both in number and in complexity, but the results of so much observation and experiment have, up to the present time, been disappointing - it may be that in seeking a sign the ardour of the workers has outstripped their wisdom.

The study of liver function has been retarded by the striking signs which failure of one function - and that not the most important one - produces, namely, the classical picture of jaundice with the staining of the integuments, the coloration of the urine, and the discoloration at times of the stools; the easy recognition of this trilogy of signs for many centuries satisfied the curiosity of the medical world, and it was not until patient pathological research showed that liver insufficiency of a degree incompatible with life could occur without jaundice, and that jaundice was frequently a manifestation, not of liver disease, but of disorders of the haemopoietic system, or of impermeability of the biliary passages, that attention became directed to the possibilities of studying other liver functions than those of bile production.

The/

The researches of many workers in many countries have placed the study of kidney function in the clinic upon a rational basis, and analogous tests of liver function are an urgent necessity; a little study will show, however, that the problems are entirely different, and that their solution cannot be expected along similar lines. It is quite possible, by means of a few simple tests, to estimate the capacity of the kidney with regard to its three great functions - urea excretion, salt excretion, and water excretion, and even to estimate its power of dealing with ingested or injected dye-stuffs - on the other hand the functions of the liver are legion, and so vital that nature has not only endowed this organ with vast reserve capacities, but it has given the extra-hepatic tissues of the organism the power, under certain circumstances, to usurp the liver's part in practically every process with which it is concerned. How, then, can it ever be possible to gain an intimate knowledge of the workings of the hepatic cellule by the use of such simple tests as the exigencies of the clinic demand?

It will be the purpose of this thesis to deal in turn with each liver function, to discuss its physiology in the light of modern knowledge, and to point out the methods which have been adopted to elucidate its functional capacity; it will be most convenient to/

to reserve the criticism of each test until the end of the appropriate section, but at this juncture a short review of the subject will be given in order to correlate what would otherwise appear to be an incohesive mass of details.

Firstly as regards liver physiology, in none of the standard textbooks on physiology to which the author has had access has a connected account been given, but crumbs of information have had to be culled from various sections; the best account is probably to be found in the *Traite de Pathologie Medicale et Therapeutique Appliquée - Foie et Pancreas* (Paris, 1923) by Brule, but this represents the somewhat too isolated French views upon the subject. From this and from other sources of information that are quoted elsewhere the following account has been compiled, and whilst it may contain matter which is of a controversial nature, it is at least a working basis for an understanding of the subject.

The liver functions may be dealt with under various headings as follows:-

(1) The Biliary Function.

This - the function whose derangement is most easily investigated - whereby bile salts and bile pigments are discharged into the intestine, has been regarded variously as an excretory and as a secretory one; the evidence is discussed at some length in the special/



special section on the subject, but a brief account will be given here.

The bile pigments are produced by the disintegration of the haemoglobin of the effete erythrocytes, and all the evidence points to the conclusion that the Kupffer cells of the reticulo-endothelial system are responsible for this; the pigments thus formed are carried to the liver, taken up by the hepatic cells, and discharged into the bile passages for excretion.

The bile salts - compounds of Cholalic acid with two amino acids taurine and glycine - are of quite unknown origin, but it is suggested that they also may be of extrahepatic origin, and that their formation has some relation to cholesterol metabolism.

Urobilin and urobilinogen are decomposition products of bilirubin perhaps formed as the result of bacterial action in the intestine, with reabsorption from the intestine and reexcretion by the liver. Evidence that urobilin can be formed in any of the body tissues is forthcoming, but knowledge is in so chaotic a state that judgment as to the correctness of any of the numerous theories must be withheld.

Uroerythrine is a brick red urinary pigment chemically related to haemoglobin and bilirubin and said to be formed when the metabolism of bile pigments is imperfect.

It/

It will thus be seen that the theory of "extra-hepatic biligenesis" as it is termed by the French school, modifies the classical views upon the biliary function, and allocates to the liver an excretory function pure and simple, analogous to that of the kidney in urea excretion.

It is clear that a gross disturbance of the biliary function requires no very special methods of investigation as the classical picture of jaundice is produced; slight degrees of liver impairment, however, may manifest themselves by modifications in biliary excretion. It is possible to estimate the amount of bilirubin in the blood serum, an increase being regarded as due either to a relative or an absolute insufficiency of the liver - relative when the blood destruction is so great that an excessive amount of bilirubin is produced, absolute when there is a normal production of bilirubin in the reticulo-endothelial system but liver is unable to excrete it. Small amounts of urobilin, bilirubin and bile salts in the urine are regarded as throwing light upon the state of the liver, and the French school especially have urged the importance of a careful urinary examination for bile and its derivatives, and it is to them that we owe the recognition of the so-called "dissociated jaundice", that is the excretion of either bile salts or bile pigments alone in various disturbances of the liver and in different degrees of haemolysis.

By/



By some the occurrence of uroerythrine or of urobilin in the urine is regarded as absolute proof of liver disorder - urobilin has been named "the pigment of a diseased liver".

## (2) The Glycoregulator Function.

Glucose is absorbed from the alimentary tract, carried in the portal blood to the liver, by means of the glycogenic function of this organ it is stored as glycogen, and when necessity arises - under the influence of both nervous and endocrine stimuli - the action is reversed and glucose is liberated into the circulation, this is the glycolytic function.

Both of these functions have been made the subject of tests - the test of alimentary glycosuria and hyperglycaemia (glycogenic function), and the test of adrenaline hyperglycaemia (glycolytic function.)

With regard to one monosaccharide the liver behaves in a peculiar manner - the ingestion of a limited quantity of laevulose causes no rise in the blood sugar content as the liver is able to store it at the same rate as it is absorbed; advantage has been taken of this fact in the laevulose tolerance test.

## (3) The Liver in Relation to Fat Metabolism.

The liver is credited with having an adipopexic or fat-fixing function, an adipolytic or fat-releasing function, /

function, and an adipogenic function whereby fat is formed from protein and carbohydrate.

Sufficient work has not been done upon this aspect of liver physiology to permit of any practical application of the facts observed.

#### (4) The Antitoxic Function.

In the process of defence of the organism against micro-organisms and their toxins, against ingested toxic substances both of an organic and an inorganic nature, and against those poisons of an endogenous origin elaborated in the alimentary tract and in the body itself the liver and kidneys play a most prominent part - that of the kidneys is almost wholly excretory and may be dismissed at the outset.

Micro-organisms in the blood stream are arrested by the liver and either killed or excreted in the bile, in certain circumstances the liver may be unable to cope with the infection and various degrees of functional impairment may arise - to mention one example the liver has been found to be fairly constantly damaged in the early septicaemic stages of enteric fever, and the familiar inflammatory conditions that occur during the course of this disease are due to the excretion of virulent organisms.

Of greater interest in the present discussion is the rôle of the liver in the various exogenous toxaemias; /

toxaemias; in phosphorus poisoning the metal is found in much larger concentrations in the liver than in any other organ in the body - the liver is endeavoring to seize all the circulating poison and in doing so it frequently suffers irreparable damage; in the toxaemias of pregnancy the absorption of the noxious products from the uterine contents results in varying degrees of liver injury from the mildest functional derangement to a universal and fatal necrosis of the hepatic lobules. These are familiar examples of the role of the liver in the defence of the organism against exogenous toxins, but this function fortunately has only occasionally to be exercised; more important still to the animal economy is the mechanism for dealing with the constant endogenous toxaemia arising in the alimentary tract.

The putrefaction of the proteins ingested is continually taking place and giving rise to a physiological intestinal toxaemia - the action of bacteria upon the amino acids such as tryptophane ends in the production of the group of aromatic toxins - indol, skatol, phenol, etc. - and these are absorbed into the blood stream and rendered innocuous by the liver; a more serious intoxication arises under conditions of pathological intestinal stasis when the flora are of an exalted virulence; the undigested proteins are decomposed/

decomposed in the small intestine and the group of ptomaines is formed - these again have to be neutralized by the liver as they are powerful nervous and cardiovascular poisons.

So important is this function that physiologists regard that peculiar intoxication of animals in whom the portal blood is diverted into the inferior vena cava by an Eck fistula as being due to the action of these unneutralised alimentary toxins; it would be possible to extend the application of this view.

A large number of tests have been used to investigate the antitoxic function of the liver.

(a) The Dye Tests.

Following the ingestion or injection of a dye the liver and kidneys share the duty of eliminating it from the body, by patient experiment those which the liver seizes for excretion by way of the bile passages have been found and used as liver function tests; after their administration the rate at which they are removed from the blood stream is determined by estimating their concentration in the blood serum at various intervals; by noting their time of appearance and their concentration in the bile after duodenal tubage, or by an analysis of the stools, or finally by examining the urine to discover whether, in the event of the liver being unable to remove them, the kidneys have been obliged to take on this function.

Among/



Among the dye tests may be mentioned the indigo carmine test, the methylene blue test, the phenol-tetrachlorophthalein test, the azorubin test, and the rose bengal test.

(b) Tests Founded upon the Detoxication of Intestinal Toxins.

The detoxication of the aromatic toxins is effected by their conjugation with either glycuronic acid or with sulphuric acid; quantitative estimations of the glycuronic acid elimination upon a fixed diet or after the administration of some member of the group, such as menthol or camphor, and the measurement of the ethereal sulphates under similar conditions serve to test whether the liver is capable or not of conjugating these bodies; if it is insufficient no rise will occur in the elimination of glycuronates or of ethereal sulphates.

(c) The Salicylate of Sodium Test of Roch.

Normally the liver should be able to destroy or at any rate to fix  $\frac{1}{2}$  grain of sodium salicylate after its ingestion by the mouth, and none should appear in the urine - if the antitoxic function of the liver is faulty some salicylate will appear in the urine. This has been used in Geneva as a liver function test.

(5) The Liver in Relation to Protein Metabolism.

The function of the liver in the metabolism of protein is an extremely complex one, and the subject/

subject is even yet not fully understood, although the perfecting of methods of analysis of the blood for its protein constituents has opened up a hitherto little explored field.

To discuss the subject even briefly is not within the scope of this introductory section, and reference must be made to a later chapter; it may be said here, however, that the investigations of the nature of the protein metabolism in various pathological conditions have proved unsatisfactory as far as throwing any light upon the functional efficiency of the liver is concerned. Conditions which are accompanied by liver injury are almost always associated with a concomitant metabolic disturbance, due to starvation, to acidosis, or to pyrexia, and even when the nature and the extent of the modifications in protein metabolism have been elucidated by biochemical analyses the investigator finds himself in a quandary as to which of the several factors at work he is going to incriminate.

Although much work has been done upon the so-called nitrogen partition tests of liver function especially in regard to the toxæmias of pregnancy the results have given the clinician so little help that he has very largely abandoned their use.

#### (6) The "Sanguine" Function of the Liver.

That the liver has some influence upon the production of those elements of the blood which procure its/

its coagulation is beyond question, and has been proved by both animal experiment and clinical observations.

The formation of fibrogen by the liver has been made the subject of the fibrogen test of liver function - in cases of derangement of the functional capacity of the organ the amount of this substance in the blood diminishes, but the technique for determining the exact quantity of fibrogen per unit volume is so complex that the test has a very limited application. The facts that have been observed, however, explain the haemorrhagic tendency that is seen in severe cases of liver necrosis. More extravagant claims have been made by certain French authors as to the clotting of the blood, the number of the platelets, the bleeding and the coagulation times, etc., in hepatic disorders, but as several of these phenomena are ascribed to disorders of the spleen (as in the so-called essential thrombocytopenic purpura haemorrhagica) their views must be regarded with suspicion.

The formation of fibrinogen, and the various other substances that play a part in the coagulation of the blood has been ascribed to this function of the liver, but as the whole question of the clotting of the blood has hardly emerged from the stage of conjecture with regard to several of the factors involved, it is not possible to elaborate any liver function tests upon so doubtful a foundation.



(7) The Thermic Function of the Liver.

On account of the glycoregulator function of the liver this organ must have a considerable influence upon the heat regulating mechanism of the body, and in some cases of acute liver atrophy the temperature has been found to be subnormal.

(8) The Bile Passages.

The presence of diseases of the bile passages and the methods for investigating them do not come within the scope of this thesis, but it may be mentioned that the use of the duodenal tube has allowed specimens of the liver and gall-bladder bile to be obtained, and both bacteriological and chemical investigations have been carried out on them. Little light has been thrown on the functional capacity of the liver in this way, but various dye stuffs are excreted in the bile, and a series of liver function tests has been founded upon this fact - the subject has been called Chromo-cholecscopy. The results of these tests have in most cases simply served to draw attention to the possibility of the estimation of the dye in the blood stream, and the methods have been a stepping stone to the study of dye retention rather than dye excretion. Some authors, however, still favour the duodenal tubage technique.

One means of testing the permeability of the biliary passages depends upon the presence of bile salts/

salts in the alimentary tract and their action in permitting the absorption of fat. By means of the ultra-microscope blood dust is visible if fat is absorbed, and in the absence of these granules it is inferred that no fat has been absorbed, and that the cause of this failure is an absence of bile salts from the digestive fluids - a state of affairs that is only seen in complete biliary obstruction.

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TESTS FOUNDED UPON BILE PIGMENT  
AND SALT METABOLISM.

Quantitative and Qualitative Tests.1. BILE SALTS.(1) In the Urine.(a) Pettenkofer's Test.

This is founded upon the fact that cholalic acid gives a profound red coloration with sulphuric acid and furfurol.

The classical test consists in the addition to the urine of pure sulphuric acid and a little cane sugar or simply furfurol (1); if the urine is rich in bile salts a purple colour is produced but it is frequently masked by the red or brown coloration which sulphuric acid produces with the diverse urinary pigments. The reaction is without either accuracy or sensitiveness.

There are various other methods of isolating the bile salts by means of ammoniacal salts or powders like animal charcoal. A review of the numerous chemical procedures will be found in a paper by Grimbert (2).

(b) Hay's Test.

This test was introduced by Matthew Hay in 1886, and it depends on a physical property of bile salts in lowering the surface tension of urine using dry flowers of sulphur as an indicator, normally they should float upon the surface of the urine but if the surface tension is/

is lowered they will sink with more or less celerity.

The value of the test has been much discussed, and its accuracy was at one time challenged on the grounds that other bodies besides bile salts could lower the urinary surface tension: pus, blood peptones, acetone, chloroform and ether after anaesthesia, sodium salicylate, potassium iodide and other drugs.

In 1910 Lyon-Caen (3) conducted a systematic research into the fallacies of the test, and came to the conclusion that normal urinary constituents had little effect in altering the surface tension. The presence of certain abnormal elements such as albumen, sugar, cholesterin, and bile pigments was shown to have little influence, but blood, peptones, alcohol, chloroform, and chloral were in Lyon-Caen's opinion possessed of this property.

In 1914 Brule & Garban (4) found that Hay's Test was possessed of far fewer fallacies than was usually believed, and that a positive test practically always meant the presence of bile salts. These authors believe that a positive test after anaesthesia or during the course of acute rheumatism treated with salicylates, or during intensive iodide treatment really means that the liver is insufficient, as the addition of these bodies to a normal urine is not enough to cause a fall of the sulphur. Pus, blood acetone, and bilirubin existing alone in a urine have not the power to produce positive results.

With/



With regard to peptonuria Lyon-Caen insisted that pneumonic patients who had shown this body in the urine a positive Hay's Test was usually found, but Brulé and Garban point out that pneumonic patients without peptonuria give a positive test, and that it is a liver disorder rather than the presence of the peptone which produces it.

In 1916 Brulé & Langevic (5) showed that the ingestion of oil of sandalwood, of cubebs, and of turpentine occasioned a strongly positive reaction with flowers of sulphur, and that these bodies are amongst the few apart from bile salts that will do this.

Brulé (6) regards the test as being the best "pis-aller" in the absence of any specific reaction, and its delicacy is extreme, detecting bile salts in a dilution of 1 in 10,000 (7).

It is necessary to take certain precautions, however; the urine to be tested must be freshly passed, and filtered to clearness; the sulphur must be carefully deposited in the centre of the surface of urine and not allowed to touch the sides of the specimen glass. Two phenomena are to be observed, the spreading of the sulphur over the surface, and the rate of descent; the latter can only be considered as an index of a positive reaction if it takes place within fifteen minutes.

It must be remembered that the test depends upon the/

the concentration and not upon the absolute quantity of bile salts present, and that a very dilute urine containing bile salts may yet give a negative result. The first morning specimen is thus the best one on which to perform the test as it is the most concentrated.

(c) Stalagmometry.

This is a very delicate method of measuring the surface tension of a liquid by calculating and comparing with a normal liquid, the number of drops per unit volume delivered by a standard burette; the technique need not be given here, as the method is too delicate for the purpose to which it is being applied. The work of Lyon-Caen (3), of Gilbert (8), and of Doumer (9) may be consulted.

(d) Oliver's Test.

This test depends upon the power of bile salts to precipitate peptone in acid solution.

Filter the urine until quite clear, acidify if necessary with a weak acid, and dilute it till the specific gravity is less than 1008. Take 60 minims of the solution in a test-tube and add to it 20 minims of the urine. If bile salts are present a decided milkiness appears at once, and is dense in proportion to the amount of acids. It may disappear on agitation, but/



but it reappears on adding more of the solution.

The test is extremely delicate, and nothing yet found in the urine interferes with it. (Hutchison & Rainy-22\*\*).

(e) Ignatowsky's Method.

Ignatowsky (31) recommends an instrument called Czapek's Capillary Manometer for finding the surface tension of a liquid, in this case the urine, and by its means the presence of 0.02% of bile salts can be detected.

(11) In the Blood Serum.

There is no method of measuring the amount of bile salts in the blood serum that is of any delicacy - Hay's test is inapplicable, and a modified Pettenkofer's reaction that has been tried by Gilbert and Chabrol (10) will detect their presence only in marked jaundice.

\*\*

Oliver's Reagent.

Powdered peptone (Savory & Moore's)	-----	$\frac{1}{2}$ dr.
Salicylic acid	-----	4gr.
Acetic acid	-----	$\frac{1}{2}$ dr.
Distilled water to	-----	8oz.

Filter until clear.

(111) In the Faeces.

(a) Triboulet (11) used a modification of Pettenkofer's method as follows:-

On to a small smear of faeces on a glass slide are dropped successively 2 drops of 20% sugar solution, and 2 or 3 drops of sulphuric acid. A brick-red colour is produced by normal bile, and in abnormal cases a more or less clear red, or no colour at all. The method is open to the same objections as is the reaction in the urine.

(b) The Haemoconies Test.

A full account of this is given in a special section q.v.

Troisier (53) whilst admitting that a lowering of the surface tension of the urine is a sign of liver insufficiency holds that other substances besides bile salts can produce the effect. He performed a series of experiments using various proteins, and he found that the surface tension of urine was lowered by peptone, polypeptides, leucine, skatol, nucleic acid, etc. He therefore puts forward the suggestion that the lowering of the surface tension is due in some cases to a disorder of the proteolytic function of the liver and not to biliary insufficiency.

1. BILIRUBIN.(1) In the Urine.(a) Gmelin's Test.

This depends upon the oxidation of bilirubin by means of impure nitric acid to the characteristic green colour of biliverdin. It is performed by layering urine over nitric acid in a test-tube, a positive result is indicated by a play of colours at the point of contact, the essential one being green.

The test is very rough and inaccurate, masked as it is by the colours given by numerous other urinary pigments: its sensitiveness is little greater than a naked eye examination of the urine.

(b) Spectroscopic Tests.

Bilirubin gives a massive absorption band in the right half of the spectrum, but here again the spectra of other urinary pigments may vitiate the accuracy of this method of determination.

(c) Trousseau's or the Iodine Test.

With a pipette 5 ccs. of urine are run under 5 ccs. of a very dilute tincture of iodine (1 part of iodine to 39 parts of 96% alcohol). Bile pigments will give a dark green ring at the junction of the liquids.

This test is open to the objections as Gmelin's *Same* but it is certainly more delicate.

(d)/

(d) Grimbert's Test (12).

To about 30 ccs. of urine a 10% aqueous solution of barium chloride is added until precipitation is complete; the precipitate is re-dissolved in 5% hydrochloride alcohol, after separation by filtration, and the mixture is heated on a water-bath for 10 minutes. The presence of bilirubin is indicated by the developments of a green coloration in the alcohol, but the addition of a few drops of hydrogen peroxide may be necessary to ensure the appearance of this colour.

(11) In the Blood Serum.(a) Method of Gilbert, Herscher & Posternack (13).

This is one of the quantitative methods, introduced in 1903, and depends upon the development of a blue coloured ring on the addition of serum in successive dilutions to nitrous and nitric acids in a series of small test-tubes. The appearance of the ring corresponds to a bilirubin content of 1 in 40,000 and from this figure the content of the undiluted serum can be worked out.

The method is open to numerous criticisms, and is only of comparative value, as the results do not correspond to those obtained by other procedures.

(b)/

(b) Fouchet's Method.

## Test Solution.

Trichloroacetic Acid	5 grammes.
Ferric Perchloride (10%)	2 ccs.
Distilled Water	20 ccs.

To 3 drops of serum 3 ccs. of the above solution are added, a precipitate containing the bile pigments, and the serum proteins at once appears, if bilirubin is present it gradually turns greenish-blue, attaining its maximum in 20 minutes. A colorimetric scale may be used for quantitative determinations.

Comparative tests with the same serum using the methods of Fouchet and of Gilbert showed the latter to indicate a bilirubin content of twice the amount of the former. (Brulé).

(c) Hymans van den Bergh's Method. (15, 16, 17 & 18).

In this section the quantitative method only will be given, as the matter will have to be reverted to when the physiology of bile pigments is discussed. (see McNee (19). )

To 0.5 cc. of the serum add 1cc. of 96% alcohol. The mixture is made in a centrifuge tube, which is then centrifuged until all the albuminous matter has sunk to the bottom and leaves a clear, yellowish supernatant fluid. If the fluid remains opaque from the presence of fatty substances these may be removed by/



by the addition of a drop or two of ether or 0.5cc. or more of alcohol.

To 1cc. of the yellow supernatant fluid is now added 0.25 cc. of Diazo Reagent\*. A violent coloration appears in the presence of bilirubin, and is maximal almost at once.

Comparison is then made with a standard solution of which there are many (McNee-ibid), either in a colorimeter or in a series of small test tubes for clinical purposes.

Van den Bergh takes for his "Unit of Bilirubin" a content of 1 in 200,000, and the content of normal human serum is 0.2 to 0.5 of a unit.

In carrying out the colorimetric test with the indirect reaction the chief point of importance is, of course, to take note of the amount of dilution of the serum necessary in the various steps of the test. Van den Bergh states that if 0.5cc. of serum is precipitated by 1cc. of alcohol, and centrifugalised, a volume of  $10/7$  is left above the precipitate. The amount of pigment lost in the precipitate is neglected, and it is taken that the bilirubin in 0.5cc. is now contained in  $10/7$  ccs. of the supernatant fluid, or is diluted  $20/7$  times. To 1cc. of this supernatant fluid is added 0.5cc. of alcohol and 0.25cc. of diazo reagent. The volume is thus increased from 1cc. to 1.75ccs. being a dilution of  $7/4$ . The addition of 0.5cc. of alcohol/

alcohol gets rid of any capacity, and also helps to bring the ultimate dilution up to a round figure.

Thus the dilution of the original serum, in this fluid which is used for the colorimetric comparison is  $20/7 \div 7/4 = 5$  times.

If in the carrying out the test the test fluid is found to be identical in intensity of colour with the standard solution (which equals "one unit" of bilirubin) then, if the dilution had been made according to the method described, the serum would actually contain not 1 but 5 units of bilirubin, having been diluted five times.

#### Diazo Reagent.

This consists of two solutions, each of which keeps well, but the mixture must be made up immediately prior to each test. The two solutions are made up as follows:-

<u>Solution A.</u>	Sulphanilic Acid	1 gramme.
	Concentrated HCl	15 grammes.
	Distilled Water	1000 ccs.
<u>Solution B.</u>	Sodium Nitrite	0.5 gramme.
	Distilled Water	100 ccs.

The Diazo Reagent consists of a mixture of these two solutions in the proportion of 25 ccs. of Solution A to 0.75cc. of Solution B.

The above account is taken from McNee's article (19).

It/



It is noted by Brule that the Van der Bergh Method gives the serum bilirubin content as twenty times as feeble as by the Method of Gilbert & Herscher.

111. In the Stools.

The Corrosive Sublimate Test.

Mix some of the stool with a concentrated solution of corrosive sublimate and allow it to stand for twenty four hours. A normal stool is turned red from the presence of urobilin; a green colour means that there is unaltered bilirubin. A complete absence of green or red coloration shows the absence of bile pigments altogether. (Hutchison & Rainy-20).

The red colour is more correctly described as being due to Hydrobilirubin (Cummer-21).

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UROBILIN.(1) In the Urine.

It is necessary to dwell at some length upon the various procedures for the detection of urobilin or its precursor urobilinogen in the urine, as so many of the tests which have been employed are inaccurate or if their accuracy is beyond reproach they may not distinguish between physiological and pathological urobilinuria on account of their great delicacy. It is essential in criticizing any researches upon the significance of urobilinuria to take notice of the method employed by the experimenter, and if this is not given, to discount or even totally discredit his conclusions.

(a) Ehrlich's Benzaldehyde Reaction.

This is a test for urobilinogen.

To several cubic centimetres of freshly passed urine a few drops of Ehrlich's reagent\* are added. If urobilinogen is present a more or less intense red colour develops in the cold.

The great simplicity of this method is its chief recommendation, but it is open to several grave objections; acetone was shown by Gavaudan (23) to give in urine a colour reaction which is indistinguishable from that given by urobilinogen itself, but fortunately there are few substances which act similarly.

A much graver objection was pointed out by Grossmann (24) and confirmed by Steensma (25), that in numerous cases the benzaldehyde reaction is negative in urine, which, after oxidation, gives a strong urobilin reaction - Grossmann found that such urine after dialysis would give a positive benzaldehyde reaction and it would appear therefore that there is some substance which actually prevents the development of the reaction.

\*Ehrlich's Benzaldehyde Reagent.

Paramethylamidobenzaldehyde	8 Grammes.
Concentrated Hydrochloric Acid	80 Grammes.
Distilled Water	200 Grammes.

The difficulty encountered in the tests for urobilin is that the true bile pigments are apt to obscure the result, and they must be precipitated by some substance which does not act similarly on urobilin - thus one may add to 10ccs. of the urine 10 drops of a 20% solution of sodium carbonate, and 10 drops of a 20% solution of calcium chloride (Steensma), or Denige's Reagent, and the filtrate will contain urobilin without other bile pigments. The urobilin is then dissolved out by means of chloroform, ether, alcohol, etc., and the final solution is examined for urobilin by means of the spectroscope or by means of its fluorescence after the addition of zinc salts.

To particularize:-

(b) Grimbert's Method.

The urine is treated with Denige's reagent\* in order to precipitate the bilirubin, etc., and is filtered. To the filtrate a few ccs. of chloroform are added, and the mixture is well shaken - the chloroform now contains the urobilin; it is decanted, and a few drops of a 1 in a 1000 solution of zinc acetate in 96% alcohol added. The urine is neutralised with several drops of ammoniacal alcohol, and the fluorescence noted; this may be obvious in daylight, or it may only appear after illuminating the liquid by means of a ray of bright light from an electric torch or a head mirror, using a dark background - thus it is possible to ascertain varying degrees of the reaction.

The test is not very delicate, and it should give a negative reaction with small amounts of urobilin such as are normally present in urine, constituting the so-called physiological urobilinuria. On this account it was used by Brulé and Garban (26) in their work upon phenomenon of pathological uribilinuria. There are losses of urobilin in the precipitate, and the solvents never dissolve out the totality of the pigment, whilst in the conversion of urobilinogen into urobilin some of the latter is certainly destroyed (27).

\*Denige's Reagent.

Red Oxide of Mercury.	50 grammes.
Sulphuric Acid.	300 grammes.
Distilled Water.	1000 ccs.

(c) Schlessinger's Method.

To 10ccs. of urine are added a pinch of powdered zinc acetate, and 10ccs. of 95% alcohol; after complete oxidation of the urobilinogen in 10-20 minutes the mixture is filtered to absolute limpidity, and fluorescence sought by means of a strong beam of light. This test, though simple, is very sensitive, and will detect the merest trace of urobilin - it is thus useful only in the study of physiological urobilinuria, and for this purpose it was adopted by Brule and Garban (28).

(c) Spectroscopic Methods.

The spectroscopic examination for urobilin (which gives an absorption band between "b" and "f") is rendered difficult by the spectra of the other urinary pigments which become superposed. Recently, however, Brandt has introduced a rough quantitative method after precipitating the foreign pigments with zinc.

Brandt's Method is as follows:- (29).

5ccs. of the urine is mixed with 5ccs. of absolute alcohol, and 50 grammes of powdered zinc acetate is added, the whole is stirred and then filtered. This process is repeated with the same amounts of zinc acetate and alcohol, but diminishing amounts of urine - the volume of the latter being made up to 5cc. with distilled water. The dilution at which the spectroscopic bands disappear is noted - normally/



normally this is with 2ccs. of urine and 3ccs. of water, a dilution of 1 in 5; this is the physiological extreme, and a positive result with a dilution of 1 in 10 is definitely pathological.

#### 11. In the Blood Serum.

This is not of very great importance in the absence of a reliable quantitative method, as urobilinuria is more easily determined.

##### Grigaut's Method. (30).

20ccs. of serum are mixed with an equal volume of distilled water and 10ccs. of the following reagent are added:-

Ferric Perchloride (French Codex)	5 drops.
10% Acetic Acid	20ccs.
Distilled Water	80ccs.

The mixture, saturated with sodium sulphate, is brought to the boiling point and filtered. The pigments are carried down with the coagulated albumens and remain on the filter-paper - the urobilin is contained in the filtrate, from which it is extracted with the usual solvents; it is then treated with zinc acetate (as in the method of Grimbert) and a fluorescence is sought.

#### 111. In the Faeces.

Urobilin in the stools is in the form of hydro-urobilin/

hydrourobilin, stercobilin, or stercobilinogen, and the detection of these bodies is fraught with many difficulties. For a full discussion of the subject reference may be made to Brule's monograph. The corrosive sublimate test is the simplest, but it is apparently impossible to detect any of the pigments in any but large amounts. The matter need not be pressed as it is of little interest in its present state of development.

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## Modern Conceptions upon the Physiology of The Bile.

### 1. THE BILE SALTS.

Whipple has well said - "If our ignorance about the complete story of the bile pigments is disturbing, then our lack of understanding as to the source and internal metabolism of the bile acids is pathetic - this in spite of much careful study and investigation".

The task of furnishing anything like a clear account of bile acid metabolism is impossible, but it is of some importance to indicate the trend of modern thought.

The bile salts are sodium glycocholate and sodium taurocholate, of which the former is both more abundant and more important; by boiling with strong acids both of the salts are split into the same acid - cholalic acid - and an amino acid glycine in the one case and taurine in the other. The amino acid constituents are the products of disintegration of the protein molecule, and are variously supposed to be derived from the red corpuscles or more probably from the alimentary tract. Cholalic acid, the more important constituent, is again of doubtful origin - from the intestine, from the haemoglobin, or from cholesterin (32).

Where the actual synthesis occurs has been the subject of much experimental work, and the hepatic cell was formerly <sup>considered</sup> to be the sight of this occurrence, and/

and the recent work of Foster, Hooper and Whipple (33) showed that in dogs with an Eck's fistula the excretion of bile salts fell by one half, but did not disappear altogether. Their experiments have, however, been criticised severely, as such interference with the animal economy as they practise would seem to invalidate the interpretation of their results in terms of normal physiology.

The classical entero-hepatic circulation of bile salts has even been criticised, as animals with external biliary fistulae can continue to pour forth these elements unabated.

The recent views point rather to an extrahepatic origin of the bile salts, having some relation to cholesterol metabolism, and the liver is regarded as having an eliminative function pure and simple; until some accurate method of dosing the bile salts in the blood is elaborated little further progress can be anticipated.

#### THE BILE PIGMENTS.

Bilirubin is the principal bile pigment; it is a definite chemical substance, having for its formula  $C^{32} H^{36} N^4 O^6$  and it is easily oxidised to biliverdin. The bile contains small quantities of related pigments, but normally bilirubin, and to a less extent, biliverdin, preponderate in the form of alkaline salts soluble in water - pure bilirubin lacking this quality.

It/

It is well established that bilirubin is derived from haemoglobin. In vitro-haematin - the ferruginous nucleus of haemoglobin - on deprivation of its iron becomes haematoporphyrine which has the same empirical formula as bilirubin, and both haematoporphyrine and bilirubin on reduction give bodies with the reactions of urobilin. So much for the actual chemistry which is a comparatively simple matter, it remains to explain where the transformation of haemoglobin to bilirubin takes place.

The old theory that this transformation occurs in the cell of the hepatic lobule can no longer be admitted, chiefly owing to the researches of Aschoff and his co-workers on the so-called reticulo-endothelial system, and of the French school on the extrahepatic formation of bilirubin. For a full account of the subject the original articles must be consulted, but critical reviews will be found by McNee (19 & 33) and by Brule (34 & 6).

Briefly the results of their experiments were as follows:-

#### THE RETICULO-ENDOTHELIAL SYSTEM.

This system was first described by Ribbert (36) in 1904 from the fact that its constituent cells took up carmine after intravenous injection; the cells include the Kuppfer cells of the liver, the endothelial cells of the spleen, bone-marrow, and the lymphatic glands/



glands, the interstitial cells of the thymus, and the capillary endothelium of the suprarenal capsules.

Subsequent researches by Aschoff (see 37), McNee, Lepehne, etc., made it certain that in conditions of excessive blood destruction the cells of the reticulo-endothelial system phagocyted intact red blood corpuscles, corpuscular debris, and haemoglobin, and they broke down the haemoglobin - it has not been actually proved whether bilirubin is formed or only a precursor, but the inference is that bilirubin itself is formed.

The French school - Widal, Abrami, Brule, Castaigne, etc., - in studying the subject from the point of view of haemolytic jaundice, have become ardent supporters of the theory of so-called "extra-hepatic biligenesis". They have shown the presence of bilirubin in pleural and ascitic effusions, in the spinal fluid after haemorrhages, and they have proved, to their own satisfaction, the local formation of bilirubin.

Whipple and Hooper (37) in the United States of America performed a series of experiments in which laked blood or pure haemoglobin was injected locally into the peritoneal cavity of dogs, and by subsequent tappings they were able to show the presence of bilirubin within twenty four hours. Further experiments along with same lines were performed in the States by/

by Pearce and his co-workers. (38).

It will be seen from the foregoing account that bilirubin is formed from the haemoglobin of the effete erythrocytes, either in the cells of the reticulo-endothelial system, or in the various cells of the fixed tissues; it arrives preformed at the cells of the hepatic lobule which regulate its passage into the biliary passages. Thus with regard to this pigment the liver would exercise an eliminative rather than secretory function.

#### The Van den Bergh Reaction.

Ehrlich (39 & 40) in 1883 and 1884 discovered that if a small amount of a diazonium salt in acid solution be added to an alcoholic solution of bilirubin coupling occurred with the development of an azo dye - azo-bilirubin. This dye was isolated in 1900 by Proscher (41) who pointed out even then that it was an excellent but neglected test for bilirubin. In 1913 Hymans van den Bergh revived the test as he considered the time propitious for the study of the blood changes in jaundice rather than the somewhat overdone examination of the icteric mucosae, stools and urine. He was able to show that icteric serum was divisible into two groups according to the manner in which it reacted to the azo test:-

- (1) Sera giving a reaction at once - Prompt direct reaction.

(2)/

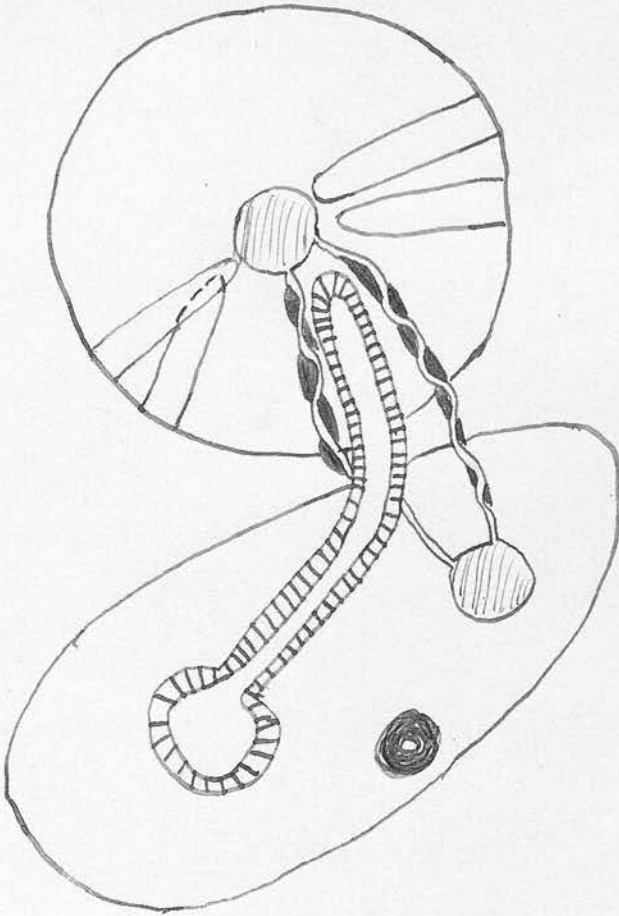
- (2) Sera giving no colour reaction, or only after long delay - Delayed direct reaction.

Every serum gives an immediate reaction with the diazo reagent after precipitate with alcohol, and <sup>this</sup> fact is made use of for a quantitative colorimetric estimation - the so-called Indirect reaction.

Freigh and Querner (42) have described a third type of reaction:-

- (3) Sera giving a prompt slight reaction, followed by gradual deepening of the colour -  
Biphasic reaction.

McNee's conception of the liver serves to elucidate the meaning of these reactions, and although it does not strictly come up for discussion under the present heading of physiology, yet it is desirable to include it in order to complete this account of the van den Bergh reaction.

McNee's Schema of the Hepatic Lobule.

To quote from McNee (Quart. Journ. Med. 16: page 362: 1923): "Each lobule can be considered schematically as made up of a series of radiating tubular glands, shaped like a test-tube, with the closed end pointing to the centre of the lobule. The polygonal glandular cells lie, as it were, along the wall of the test-tube, which may be taken to represent a basement membrane. In the centre of the test-tube surrounded entirely by the polygonal cells, lies the bile capillary. Between the tubular glands run the wide portal vascular capillaries, passing from the portal tract to join the branch of the hepatic vein in the centre of the lobule. Along the walls of these capillaries lie a number of large endothelial cells (Kuppfer cells)".

Jaundice might arise in several ways.

- (1) When the preformed bile pigment passes the glandular liver cells, but is obstructed in the bile passages, it is reabsorbed into the blood stream and gives a prompt direct reaction.
- (2) Where, owing to damage and functional derangement of the polygonal cells the bilirubin is unable to enter them; it passes direct to the hepatic vein and gives a delayed direct reaction.

A subvariety is due to such massive haemolysis that bilirubin is formed in too great a quantity to be dealt with by the polygonal cells and it passes into the hepatic vein in part.

(3)/



- (3) Where in addition to damage to the polygonal cells there is obstruction in the bile passages. Under such circumstances some bilirubin might pass direct to the portal vein (giving a delayed direct reaction), and some pass through those polygonal cells which were still functioning, to be obstructed in the biliary passages, and reabsorbed (giving a prompt direct reaction).

In this way a Biphasic reaction would be accounted for.

It will be seen from this view than van den Bergh's reaction is explained by some alteration taking place in the bilirubin during its passage through the polygonal cells, which facilitates the development of the colour changes of the diazo reaction, and produces a prompt direct reaction.

### 111. UROBILIN.

The physiology of urobilin has been the cause of much experimentation and of much dispute, so that it is impossible to give a definite statement of the accepted views; a list of the theories will therefore be set out.

#### (1) The Hepatic Theory.

For Hayem and his pupil Tissier (43) urobilin is a special pigment secreted by the injured liver cell, manufactured/

manufactured in place of bilirubin from haemoglobin, either because the liver cell is unable to perform its normal pigmentary function, or because, in certain haemolytic conditions it is overwhelmed by more haemoglobin than it can deal with, and is thus relatively insufficient. There are numerous objections to this theory - in many cases of grave hepatic lesions marked bilirubinaemia may be observed in the absence of urobilinuria, and the clinical data which could be quoted as incompatible with Hayem's theory are many; the theory would appear to be untenable on other grounds, however. Urobilin is not a more primitive pigment than bilirubin but a body which is necessarily a product of its disintegration, and its presence postulates the previous formation of the true bile pigments.

## (2) The Renal Theory.

To Gilbert and Herscher (44) this theory is in the place first<sup>due</sup>, as their work first put it upon a sound basis. In cases of hyperbilirubinaemia the kidney has the power of transforming bilirubin into urobilin, but in very marked cases the kidney is, - as it were, overwhelmed by the bilirubin and its function of conversion is paralysed so that bilirubinaemia occurs in the absence of urobilinuria. They regard urobilinuria as an index of biliary retention, and not of liver insufficiency.

This/

This theory has become untenable since it has been proved that urobilin may be present in the serous cavities in both normal and abnormal conditions. It also has been found in the blood stream in the serum - it actually circulates in the body, and the kidney is merely the medium of filtration and of excretion.

(3) The Tissue Theory.

Certain authors - Kunckel, Cordua, Quincke, Glasens (45) suggest that the conversion of bilirubin into urobilin may take place in any tissue.

(4) The Haemic Theory.

It has long been known that haemoglobin may be converted by stages into haematin, haematoporphyrine, and finally into urobilin; Hayem in 1887 suggested that in conditions of haemolysis part of the haemoglobin became bilirubin and part of it urobilin. More recent work on haemolytic jaundice has proved that following the injection of haemolytic agents one is able to demonstrate the appearance, with small doses - urobilinuria, with larger doses - urobilinuria and cholaluria, and with massive doses - haemoglobinuria; further in most cases of haemolytic jaundice urobilinuria is the rule and choluria the exception.

It must be admitted that the haemic theory is in part correct, and that urobilin can arise in this way.

(5) The Intestinal Theory.

As/

As the bilirubin which reaches the intestine by way of the bile ducts is completely transformed there into stercobilin, numerous authors since the time of Muller have argued that the urobilin which appears in the urine is all formed in the intestine. This view is somewhat difficult to understand as urobilinuria is essentially a symptom of bile retention, in which the amount of bilirubin reaching the intestine is diminished; to invoke the explanation of "pleiochromia" or abnormal thickness of the bile so that a reflux takes place into the blood stream from the bile capillaries and so causes jaundice, whilst the remainder passes into the intestine so rich in bilirubin that an abnormal amount of urobilin is formed is not looked upon as a satisfactory argument.

#### (6) The Entero-hepatic Theory.

This is the theory which at the present day has the largest number of adherents. Fischler (46) holds that the urobilin formed in the intestine is normally absorbed in part into the portal circulation, carried back to the liver, and re-excreted, there being an enterohepatic circulation of urobilin as of bile salts. If the liver cell is damaged, re-excretion does not take place completely, and urobilinuria to a greater or less extent occurs; further in the presence of marked biliary obstruction no urobilin can be formed in the intestine as bilirubin is absent, and urobilinuria does not result.

To/



To a certain extent, therefore, clinical findings are confirmed. Brulé in his monograph gives an exhaustive critical review of the subject and arrives at the conclusion, that, tempting as the theory is, it cannot be accepted. For a full discussion his work must be consulted, but several of his arguments may be mentioned.

In the newly born baby stercobilin is absent from the stools, the pigment being entirely bilirubin or biliverdin, but nevertheless urobilinuria is constant; again in haemolytic jaundice where there is no question either of hepatic insufficiency or of diminished bile excretion urobilinuria is present to a marked degree - these clinical facts are supported by ample experimental data.

#### (7) Brulé's Personal Theory.

Urobilinuria is the consequence of hyperbilirubinæmia, and as the renal threshold for this body is high in man the transformation into a more diffusible and less toxic substance takes place - urobilin is formed. Brulé believes that this transformation may take place in any tissue. If the bilirubin in the blood reaches too high a level both it and urobilin appear in the urine, whilst at still higher levels bilirubin alone is excreted either because its modification is no longer necessary or because the tissues, saturated with bile, have lost their power of conversion.

Physiological/



Physiological urobilinuria merely corresponds to physiological bilirubinaemia.

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It will thus be seen that many theories have been promulgated to explain the occurrence of urobilinuria, and whilst a criticism of them is out of place it may be urged that Brulé's theory is more in consonance with the recent views of the extra-hepatic origin of both bile pigments and bile salts, and that it suffers from few of the obvious inconsistencies of its predecessors.

Whichever theory be adopted it seems to have been universally shown that urobilinuria is a sign of a diseased liver if it occurs to any extent, and that its detection in any but the smallest amounts will give rise to the conclusion that there is a hyperbilirubinaemia, due either to a damaged liver or to excessive haemolysis. In the presence of marked bilirubinuria the disappearance of urobilinuria may be expected.

#### (IV) STERCOBILIN.

Bilirubin is delivered into the intestine, and during its transit through the alimentary tract it is converted by the action of bacteria or of enzymes into less complex pigments of which one is stercobilinogen, a body closely related chemically to urobilinogen, and oxidised/

oxidised similarly to stercobilin or hydrourobilin or faecal urobilin. It is certain that numerous pigments are formed, either intermediate between bilirubin and stercobilinogen or even more elementary than this latter body.

It seems to have been proved that even in the presence of a complete biliary obstruction stercobilinogen continues to appear in the stools, and it is suggested by Brule that this is a mode of ridding the organism of excess of bile pigment, by its excretion as urobilin through the intestinal walls. Under certain conditions bilirubin can appear in the stools, physiologically in the new-born, and pathologically owing either to excessively rapid transit of the intestinal contents, or to a greatly increased excretion of bile as in cases of excessive blood destruction.

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CLINICAL CONSIDERATIONS.

MacCormac and Dodds (47) in an investigation into the effects of neosalvarsan treatment of syphilis on the liver function used a number of tests of liver function, chiefly, however, those for the disturbance of the bile salt and pigment metabolism. In the blood they used Fouchet's test for hyperbilirubinaemia, and Lowenhart's test for blood lipase; in the urine they used Gmelin's, Hay's and the iodine tests for bile salts, and pigments, and Schlessinger's and Erlich's tests for urobilinuria. Their results will be tabulated.

Case	Blood			Urine			
	F.	Lip.	Ehr.	Uro.	Iod.	Hay.	Gmelin.
Cancer of gallbladder	+	.6	+	+	+	+	+
Cirrhosis Jaundice	+	.5	+	+	+	+	+
? Cirrhosis Jaundice	+	.6	+	+	-	+	-
Early cir. jno. Jaund.	+	.5	+	+	-	+	-
Hanot's Cirrh.	+	.7	+	+	+	+	+
Ac. Catar. Jaundice.	+	.5	+	+	-	+	-
Biliary Obstr. Jaundice.	+	.4	+	+	+	+	+
Ac. Catarr. Jaundice.	+	.5	+	+	-	+	-

After ascertaining the accuracy of the tests upon a series of positive controls the authors examined 57 syphilitic cases at different stages of the disease and of treatment, they were able to divide the cases into two groups:-

(a) Positive cases:-

- (1) Dermatitis      (2) Jaundice      (3) Tests positive -  
Clinically negative.

(b) Normal - the majority.

Litzenberg (48) made an inquiry into liver efficiency in normal pregnancy, and he regarded the presence of urobilin or urobilinogen in the urine as the only test of liver function at that time of any practical value. The view he adopted as to the origin of these substances is as follows:- Haemoglobin is broken down by the Kupffer cells, passes to the liver and is converted into bilirubin, and thence it is excreted in the bile. In the intestine the bilirubin is decomposed by the action of bacteria to form urobilinogen, this body is absorbed, passes to the liver where it is retransformed into bilirubin and re-excreted. If the liver be functionally insufficient this retransformation of the urobilinogen does not take place and urobilin appears passes into the blood to be excreted in the urine. Urobilinuria and urobilinogenuria would thus indicate (1) that bile is reaching the intestine, and if it is very marked (2) that/



that the liver is insufficient either absolutely or relatively as in conditions of great haemolysis.

In his work he used Ehrlich's test for urobilinogenuria and that of Schlessinger for urobilinuria. He tested 71 normal non-pregnant persons and found that none of them showed the presence of either of these bodies in the urine. In 200 normal pregnant women he found that 31% gave a positive result. He attributes this to minor degrees of liver insufficiency due to pregnancy.

In an extensive research into the value of liver function tests lasting for a period of two years, Dedichen (49) came to the conclusion that urobilinuria was a constant sign of liver disease when the bile passages were permeable, and primary anaemia was excluded.

Felsenreich and Satke (50) compared the findings in regard to bilirubin and its derivatives in serum, duodenal fluid, stools and urine in 38 cases of simple jaundice. Abnormally high urobilinogenuria they found was a constant sign of liver disease, and the degree of liver impairment can be estimated by comparing the urobilinogenuria and the elimination of bilirubin in the duodenal bile.

Piersol and Bockus (51) found in a series of 14 cases that urobilin was present in the urine by Schlessinger's method in all cases which showed by Rosenthal's test a marked hepatic insufficiency; in cases/



cases of slight impairment urobilinuria may be absent.

Crainicianu and Popper (52) studied the liver function in 47 cases of normal pregnancy in the last month - their findings with regard to the biligenic function of the organ will be given here and those with regard to Widal's haemoclastic in the appropriate section.

- (a) Bile salts in the urine in 20% of the cases by Hay's method.
- (b) Pigments in the urine in 8% of the cases by Grimbert's method.
- (c) Urobilin in the urine in 12% of the cases by Deniges' method.
- (d) Albumen in the urine in 28% of the cases.

They attribute these facts to a mild impairment of the liver in normal pregnancy.

Filinski (54) introduced a modification of the urobilin test, he gave the patient 100 grammes of glucose or 150 grammes of honey in 300ccs. of tea on a fasting stomach, and observed the urine in two hour specimens for the succeeding 12 hours, during this time no proteins were taken. In 30 cases of affections of the liver there was an increase in the urobilinogen in the urine after 4-10 hours. Among 35 controls only 5 were positive, and even in these some disturbance of the liver function was probable.

A record of the findings in over 70 cases with regard to urobilin, bilirubin, and bile salts will be found/

found in the section on the sodium salicylate test.

### The Van den Bergh Test.

Gerrard (55) studied the blood bilirubin in 370 cases of syphilis undergoing treatment with neo-salvarsan. The Van den Bergh test was applied to all of these cases on admission, and again at intervals during the course of the treatment.

In 281 cases no increase in bilirubin was found at any time. In the remaining 89 cases the bilirubin content of the serum was definitely increased, and in 67 of these on admission the number of units was less than one; in 22 cases on admission the bilirubin showed a definite increase, ranging up to 2.5 units, and of these 17 were unaffected by further treatment, but the remaining 5 showed a very definite increase after additional injections.

Of the 67 cases which on admission had no increase in the bilirubin of the serum, treatment with N.A.B. produced the following changes:-

21 cases showed an increase to 1 unit of bilirubin.

24	"	"	"	"	"	1.5	"	"	"
13	"	"	"	"	"	2	"	"	"
3	"	"	"	"	"	2.5	"	"	"
6	"	"	"	"	"	3	"	"	" or over.

Of these last six cases, who were regarded as suffering from latest jaundice, four soon developed definite/

definite icterus, and it was noted that the coloration of the skin and the mucosae occurred with a bilirubin titre of 4 units.

The author considers the Van den Bergh test as the simplest and reliable one that the syphilologist has at present, and it was instrumental in averting in his cases the development of severe hepatic toxaemias.

In an assorted series of 184 cases Andrews (56) investigated the value of the Van den Bergh reaction - his cases were chiefly jaundice. His main conclusions are:- The test is of value in detecting latent jaundice, i.e. bile retention before it reaches such a degree as to cause icterus. It enables us to detect the presence of a haemolytic process in the body, and thus to distinguish between a cancerous or other secondary anaemia and pernicious anaemia. It settles whether a yellow pigmentation of the skin or serum is really due to bilirubin. It is of considerable value in determining whether a haemorrhagic fluid is due to recent bleeding, e.g., at the time of puncture or not. All cases of obstructive give a direct, all cases of haemolytic jaundice in indirect. Jaundice due to liver cell damage may give a direct or an indirect reaction, hence in the group of cases which give rise to greatest clinical difficulty it may be of no value. It is of no value in distinguishing catarrhal from obstructive jaundice.

### CRITICISM OF THE TEST.

A very considerable amount of space has been devoted to the various tests which depend upon the biliary function of the liver, as they furnish us with a number of simple procedures throwing light upon the efficiency of the biliary function. It would be unwise to belittle the value of the results because they are attainable by simple means, and it is felt that a rational understanding of the lessons that can be drawn from a few simple urinary and blood analysis will render, in many instances, the use of the more elaborate tests of liver function as unnecessary as they are tedious.

To deal, then, with each of the biliary constituents in turn:-

#### (1) In the Urine.

##### (a) Bilirubin.

The presence of bilirubinuria indicates (a) a disturbance of the function of the liver cells, (b) biliary obstruction, or (c) an excessive haemolysis.

In the absence of clinical jaundice the factor of biliary obstruction can be excluded, but the elimination of the role of haemolysis is not so easy, especially when it is necessary to distinguish between mild hepatic dysfunction and mild haemolysis.

Urobilinuria is always found to occur at an earlier/

earlier stage in the disease - be it haemolytic or hepatic - than bilirubinuria, and the estimation of the former is a more delicate procedure than that of the latter, because it is provoked by a smaller functional disturbance. The use of bilirubinuria as a test is therefore not of very much importance.

(b) Urobilin.

If care is taken to select a test for urobilinuria that is not sufficiently delicate to give a positive result in the presence of a physiological urobilinuria the test is of very considerable value. It is subject to the same objections as the test for bilirubin, as the existence of a haemolytic process in the body will provoke an excretion of urobilin in the urine, and it is stated that in complete obstruction of the biliary passages no urobilin can be formed. While it is true that in the cases in one series which gave a positive Rosenthal's test there was also urobilinuria, and a great wealth of other clinical data confirm the fact that urobilinuria is an almost invariable accompaniment of liver disease of a moderate degree, the converse is not true, and it cannot be said that every case showing the presence of urobilinuria has an insufficient liver.

(c) Bile Salts.

Hay's test for bile salts in the urine is one of the most valuable of the simpler procedures for the study/



study of the liver efficiency. Haemolysis does not appear to have any relation to bile salt metabolism, and the only fallacy to be excluded is the presence of biliary obstruction. A full discussion of the subject is to be found in an earlier part of this section, and all that need be done here is to recommend the test as a routine measure in the examination of all suspected liver cases, and to regard it as being midway between urobilinuria and bilirubinuria in delicacy.

(2) In the Blood Serum.

(a) Bilirubin.

The use of the test introduced by Van den Bergh as a means of distinguishing between the different varieties of jaundice has already been dealt with, and it is the interpretation of the quantitative estimation of the bilirubin in the serum in cases of suspected liver dysfunction that is of importance to the present issue.

Gerrard's work showed the value of such an estimation in the treatment of cases of syphilis with N.A.B., but he was able to create conditions which would not be available in the ordinary course of events in the clinique. He was able to ascertain the bilirubin titre of the sera before the beginning of treatment, and he had a control in each case. In the clinique the figure that would be obtained in a case of liver/

liver disease would have to be compared to the normal standard and the control conditions of Gerrard could not be reproduced. A further grave objection is the question of haemolysis which is not distinguished by the quantitative estimation.

It can only be concluded that Van den Bergh's test is applicable to conditions such as obtained in Gerrard's series.

(c) Urobilin.

There is no accurate method for determining the urobilin content of the serum, and even if there were the question of haemolysis would complicate the tissue.

(d) Bile Salts.

As bile salt metabolism is not dependent upon blood destruction it would seem that an accurate method of estimating their amount in the serum would solve to a great extent the problem of liver function testing, and furnish, at least, an index of the capacity of the liver with regard to its biliary function.

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THE METABOLISM OF PROTEIN.

INTRODUCTORY.

The ingested proteins are digested in the alimentary tract to the soluble amino-acids, and there is much evidence to prove that it is only these bodies that are absorbed:-

- (1) Slowly developing anaphylactic reactions follow the injection of proteins similar to the blood proteins.
- (2) Fatal results follow the injection of foreign proteins such as snake venom, etc.
- (3) During the digestion of a protein meal it is impossible to detect foreign protein in the blood even by precipitin reactions.
- (4) Very slow injection of perfectly digested protein does not result in any untoward reaction thus showing a complete reduction to aminoacids.
- (5) Amino-acids can be separated from the blood serum during digestion.

The protein molecule is broken down into its ultimate building stones, the amino-acids, by the digestive enzymes of the alimentary tract. These amino-acids are absorbed into the blood, by which they are carried to the various organs and tissues which sift/

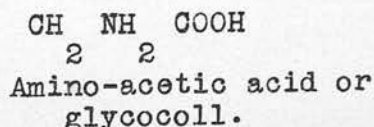
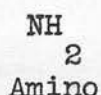
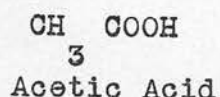
sift out the amino-acids which they require for the reconstruction of their broken protein. The acids not required for this purpose, along with those that may be liberated in the tissues themselves by the disintegration of the tissue proteins are then split into two portions, one represented by ammonia, and the other by the remainder of the amino-acid radicle. The former is excreted as urea, and the latter is oxidised to produce energy.

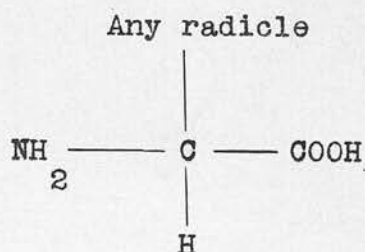
### THE CHEMISTRY OF PROTEIN.

Proteins are formed of numerous smaller molecules - the amino-acids - and the breaking down of the links of the chain is effected by the taking up of a molecule of water at each point of disruption. This process of hydrolysis is effected by mineral acids, by alkalis and by specific enzymes.

#### The Amino-Acids.

There are 18 different amino-acids, but they all have a similar structure with the presence in each molecule of (1) an amino group with a basicity comparable to that of ammonia and (2) an acid group with an acidity comparable to that of acetic acid:-



General Formula of any Amino-acid.

By virtue of the COOH group they can act as acids, or as alkalies with the NH<sub>2</sub> group.

Van Slyke's test for the presence of amino-acids depends upon the setting free of nitrogen by means of nitrous acid, and its measurement in a simple apparatus; it is both quick and accurate.

Protein Synthesis.

The amino-acids unite with each other to form peptides, the amino group of one uniting (with the exclusion of water) with the carboxyl group of the next, as the compound still has a free amino and carboxyl group at each end the process can be repeated to produce peptides, tripeptides, and polypeptides; a polypeptide containing 18 amino-acid groups has been synthesised.

There are three groups of amino-acids:-

Acid.

(1) This contains glutamic and aspartic acids - the acidity resembles that of acetic acid.

(2)/

(2) Basic.

There are three in this group - they occur in the protamines.

(3) Neutral.    The largest group.

- (a) Containing aromatic or benzene rings - tyrosine and tryptophane.
- (b) Containing the pyrrolidine ring.
- (c) Containing the aliphatic chain or fatty acids.

The Amino-Acids in the Blood.

The amino-acids have not been separated in a pure state from the blood, but by the method of vividiffusion - this is performed by tying a long collodion tube immersed in water between the two cut ends of an artery of an anaesthetised cat - the amino-acids diffuse into the surrounding liquid and they can be separated in a very dilute solution. By the method of Van Slyke the fasting blood contains 3 to 5mg.% of amino-acids, and this figure is almost doubled during the digestion of a protein meal.

The Amino-Acids in the Tissues.

After entering the circulation the excess of amino-acids very quickly disappears from it again; after injecting 12gms. of alanine into the vein of a dog 90% was found to have disappeared in 5 minutes. The tissues absorb the amino-acids according to their ability, thus there is 60-80% increase in the muscles, and 125-150% increase in the liver, and the absorption proceeds/



proceeds until some equilibrium is attained with the blood at 3-10 mg.%. The great part of the amino-acids is stopped by the liver - in 24 hours they have been largely excreted as urea, and it would appear that during digestion they are disposed of at the same rate as they are absorbed.

#### The Fate of the Amino-acids.

The end product of amino-acid is urea and the liver apparently has the function of seizing the excess and converting it into urea. For example after a protein meal the blood urea rises markedly, in one experiment from 10mg% to 18mg% after one hour and 25mg% after two hours. The amino-acids absorbed by the extra-hepatic tissues are quickly converted into formed proteins, which are later broken down during metabolic processes to liberate amino-acids again, and these are dealt with in the same way as those of exogenous origin.

The liver is thus responsible for converting much of the amino-acids into urea, but the tissues can also perform the same function.

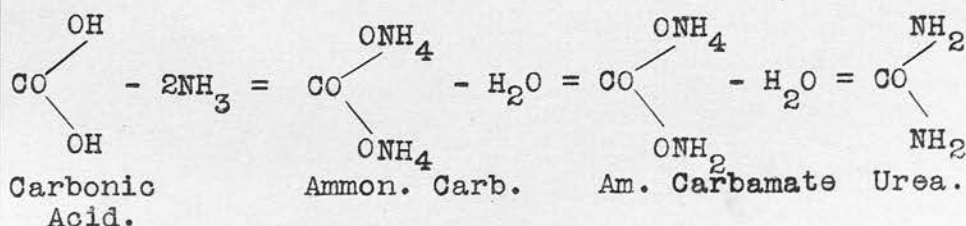
There is little reserve of amino-acids in the body, and during starvation the muscles are found to be richer in amino-acids than at other times, indicating that a transfer of amino-acids from the non-essential tissues to supply the needs of the vital tissues is occurring.

### THE END PRODUCTS OF PROTEIN METABOLISM.

These include urea, ammonia, creatinine, creatine, amino-nitrogen, undetermined nitrogen, purine bodies, and sulphur.

#### Urea and Ammonia.

During the intermediary metabolism of the majority of the amino-acids the amino group is broken off as ammonia, which combines with the available acids to form neutral ammonium salts; the most available acid is carbonic acid, so that ammonium carbonate is formed (a little ammonia unites with chlorine radicles to form ammonium chloride which is excreted as such). The ammonium carbonate becomes quickly converted into ammonium carbamate, and then urea. The process of urea formation may therefore be considered as having the function of preventing the accumulation of ammonium carbamate - a noxious substance - and thus is a detoxication process.



On an ordinary diet a man excretes somewhat more than 90% of his total nitrogen as urea, and about 3% as ammonia, the remainder of the nitrogen appearing as/

as other nitrogenous metabolites.

The influence of Acidosis on the Ammonia-Urea Ratio.

A large proportion of the ammonia is used to neutralize abnormal acids in the organism - the ammonia being probably produced in the kidney. When mineral acids are given, or when endogenous acids are produced the ammonia excretion in the urine rises immediately, as in diabetic and other forms of acidosis. Ammonia in these cases is to be regarded as an alkaline reserve, but an increase in ammonia nitrogen does not postulate acidosis, as it occurs in certain conditions in which the alkaline reserve of the blood is within normal limits, hyperaemesis gravidarum is an example of this.

In alkalosis the ammonia excretion becomes decreased, as in taking alkali after food, and in the alkalosis produced by forced breathing.

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THE INFLUENCE OF THE LIVER ON THE AMMONIA-UREA RATIO.(1) Removal of the Liver.

There are several facts that indicate that other causes than acid production may interfere with the conversion of ammonia into urea. It is natural to assume that liver dysfunction will cause less ammonia to be converted and that the ammonia co-efficient will rise. Observations to prove this have been carried out (1) after partial or total removal of the liver and (2) by perfusing the liver outside of the body.

Eck's Fistula is a union of the portal vein to the inferior vena cava so that the portal blood passes straight to the inferior vena cava and the liver is supplied only by the hepatic artery. The animals live for a considerable time, and the ammonia co-efficient frequently rises, but not to such an extent as might be expected if the liver were the sole seat of urea production. If much flesh food is given to the animals a toxic condition with stimulation of the central nervous system arises, due either to ammonium carbamate or to other metabolites. If in an animal with an Eck fistula the hepatic artery is tied a more pronounced increase in ammonia excretion takes place during its short remaining life, the blood sugar falls rapidly.

When the liver is perfused urea gradually accumulates/



accumulates in the fluid, particularly after the addition of ammonium carbonate; when other viscera are perfused no urea is formed. There are many objections to the acceptance of the results of perfusion experiments.

### Clinical Considerations.

Since the liver is an important seat of urea formation the question arises as to whether the relative percentage of urea and ammonia in the urine will become affected by hepatic disease - the results of observations are not striking. In extreme destruction, such as in phosphorus poisoning, there may be a great increase in the ammonia coefficient and in acute liver atrophy it may reach 70%.

In mild cases - cirrhosis, etc., - the results are not marked, and if an increase occurs it may be very well due to acidosis.

### The Amino-Acids.

Little difference is found in the amino-nitrogen in experimental chloroform and phosphorus poisoning until the later stages. In man small amounts of amino-nitrogen have been found in cases of impaired liver function. In eclampsia Losee and Van Slyke have shown that the amino-acids are not increased in the blood or urine. The role of the kidneys in preventing urea excretion is to be remembered in cases of nephritis.

### Creatinine/



### Creatinine and Creatine.

Creatine and creatinine are very largely products of endogenous metabolism, although some of the creatine and creatinine present in the food may appear as creatine in the urine.

### Essential Chemistry.

Creatine is methyl-guadinine-acetic acid, and creatinine is its anhydride. Quantitative estimations of creatinine may be made by the method of Folin, and it is possible to estimate the amount of creatine indirectly.

The metabolism of these bodies is obscure, but from a vast amount of work certain facts have emerged. The average daily excretion of creatinine is about 25mg. per kilo body weight in a lean person, and 20mg. per kilo in a fat person, so it would appear that creatinine excretion is dependent upon muscle mass, a fact that is confirmed by the fact of its diminution in the muscular dystrophies.

Although creatine and creatinine are largely products of endogenous origin, a certain amount of them is due to their assimilation in the food; it is well established that the bulk of the ingested creatinine is excreted in the urine. Creatine excretion is more complicated, it is present in the urine of children to a considerable amount, but in the urine of adults there is only a trace; in women it appears at/

at the menses, during pregnancy and the puerperium. Fed to starving animals creatine is not excreted, and there is evidence to suggest that it is used directly in the anabolic processes of the muscles.

Starvation causes a return to the infantile ratio of creatine and creatinine, and the administration of a semi-starvation diet with complete absence of carbohydrates has a similar effect. Estimations of total creatine - creatinine excretion under these circumstances, show little divergence from the normal amounts.

#### The Origin of Creatine and Creatinine.

The knowledge on this subject is very scanty, and it is impossible to furnish any very coherent account. As regards the distribution in the body; the voluntary muscles contain the largest proportion, the heart a medium proportion, and the involuntary muscles a relatively small amount; the liver and other glands contain traces, and the blood  $1\text{mg}\%$  of creatinine and  $3\text{mgs}\%$  of creatine. In all of these places the greatest proportion of the creatine-creatinine exists as creatine, the reverse of what occurs on excretion; this transformation of creatine to creatinine is probably carried out by an enzyme, and both the blood-serum and the liver have been shown to contain one.

The origin of creatine is quite unknown, but it may have some relation to the formative metabolism of muscle./

muscle. It appears in the urine in phosphorus poisoning, in cancer of the liver, and in the puerperium, even in the absence of the uterus as in cases of Caesarean-hysterectomy. Its elimination is not a sign of cellular destruction, and muscular fatigue leaves the creatinine content of the muscles unchanged. In the late stages of nephritis the creatinine accumulates in the blood, and serves as an index of the gravity of the condition.

#### THE UNDETERMINED NITROGEN AND DETOXICATION PRODUCTS.

##### The Undetermined Nitrogen.

Included under this heading are amino-acids, peptides, and basic substances; the amount of amino-acids and peptides in health is small, but in liver disease leucine and tyrosine may appear in fair quantity. The peptide is known as oxyproteic acid, and it becomes increased in phosphorus poisoning, and in conditions accompanied by excessive protein metabolism. The basic substances are of the nature of ptomaines, and probably have a similar origin - to them the toxicity of the urine is mainly due.

##### The Detoxication Products.

Certain noxious substances are produced in the intestine during the digestive process, and others result from the metabolic processes in the tissues.

To/

To guard against the harmful action of these substances on the organism they become detoxicated in various ways, mainly by forming inert compounds with other substances, particularly with glycocoll, sulphuric acid and glycuronic acid. The compound thus formed is excreted in the urine.

### Hippuric Acid.

The detoxication of benzoic acid, derived from certain vegetable food and fruits, and from canned foods in which it is used as a preservative, is effected by its union with glycocoll to form hippuric acid. This body is excreted in large amounts by herbivorous animals, but in man its excretion is minimal unless a <sup>diet</sup> vegetarian<sub>is</sub> indulged in, but it undergoes important variations in disease. The synthesis takes place in the liver and kidneys.

### The Ethereal Sulphates and Glycuronates.

The other substances used for detoxication purposes are sulphuric and glycuronic acids; phenol and cresol - products of the bacterial decomposition of protein in the intestine - are absorbed, and in the liver are conjugated with sulphuric and glycuronic acids to form sulphates and glycuronates. The aromatic sulphate further combines with potassium, and is excreted as the so-called ethereal sulphate; another ethereal sulphate is indoxyl sulphate of potassium or indican./

indican. Indol is absorbed, oxidised to indoxyl, conjugated, and excreted as indican. Skatol undergoes a similar detoxication. The estimation of indicanuria is a fairly reliable guide to the extent of the intestinal putrefaction, but a tissue infection, such as abscess formation, may cause hyperindicanuria.

A description of glycuronic acid is given in another section.

#### URIC ACID AND THE PURINE BODIES.

The chemical nature of the purines is well understood, and from the following table the relation of the various members of the group will be clear. On oxidation of purine the first oxidation product is hypoxanthine, and adenine is the amino derivative of hypoxanthine; similarly xanthine is the second oxidation product and guanine is its amino derivative; uric acid itself is the third oxidation produce.

<u>Purine</u>	$C_5H_4N_4$		
<u>Hypoxanthine</u> ( <u>Monoxypurine</u> )	$C_5H_4N_4O$	---- <u>Adenine</u> ( <u>Amino-purine</u> )	$C_5H_4N_4NH_2$
<u>Xanthine</u> ( <u>Dioxypurine</u> )	$C_5H_4N_4O_2$	---- <u>Guanine</u> ( <u>Amino-dioxypurine</u> )	$C_5H_4N_4O_2NH_2$
<u>Uric Acid</u> ( <u>Trioxypurine</u> )	$C_5H_4N_4O_3$		



In addition to the purines of animal origin there are others of vegetable origin - the methyl purines - which exist as the alkaloids of tea and coffee, namely, caffeine, theobromine, and theine.

#### The Chemical Nature of the Purine Compounds in the Body.

In general it may be said that the aminopurines - adenine and guanine - together with the pyrimidine bases - thymine and cytosine - occur combined with phosphoric acid and a carbohydrate in the various nucleic acids, each of which is combined with some simple protein to form nuclein, the essential constituent of the chromatin of the cell nucleus. Nucleic acid may be considered as a compound of polyphosphoric acid, containing carbohydrate groups, which serve to link the phosphoric acid molecules to the purine and pyrimidine.

#### The History of the Nucleic Acid in the Animal Body.

The breaking down of the nucleic acid is carried out by various enzymes; it is first split by the nucleases into simpler nucleotides, two of purine and two of pyrimidine from each nucleic acid molecule. Each nucleotide molecule may be decomposed in one of two ways (1) either by the splitting off of the phosphoric acid, leaving guanosine or adenosine, or (2) by the splitting off of both the phosphoric acid and the carbohydrate molecules leaving free purine bases - the enzymes responsible for this are phosphonuclease and/

and purine-nuclease respectively. The next step in the decomposition process is the splitting off of the amino group to produce the corresponding oxypurine, and a special series of deaminizing enzymes are known. The joint action of these enzymes leads to the formation of the oxypurines, xanthine and hypoxanthine, which are oxidised to uric acid by xanthine-oxidase.

The distribution of the various enzymes in the body is of interest. The nucleases are absent from the gastric and pancreatic juices, but present in the intestinal juice and the intestinal mucosa. The other enzymes are present in the kidney, lung, liver, spleen and pancreas, with a somewhat different distribution in the various species of animals; for example guanase is absent from the tissue of the pig, and deposits of guanine may occur giving rise to guanine gout; in man adenase is absent from all the organs.

Xanthine-oxidase exists only in the liver.

In most of the lower animals uricase is present, and it changes uric acid to allantoin, so that the ingested uric acid cannot be recovered unchanged from the urine. Uricase is, however, absent from the tissues of man, and this fact places him in a unique position as regards the metabolism of nucleic acid, and renders the investigation of the problem particularly difficult, since experiments upon the usual laboratory/

laboratory animals are useless. More recently it has been shown that the nucleic acid metabolism of the Dalmation dog is very similar to that of man and a more hopeful field of investigation has been opened out.

The main purine excretory product in man is uric acid, but there is also a certain amount of purine bases; the accepted view is that the purine is partly exogenous and partly endogenous. In experiments it has been found that the uric acid excretion accounts for less than half of the purine nitrogen ingestion, and it is not known how the body deals with the purine that disappears.

#### The Source of the Endogenous Purines.

There are two possible sources from which the endogenous purine excretion in man may arise (1) by the synthesis of two urea molecules or (2) by the oxidation of the lower purines.

#### Synthesis.

In birds and reptiles practically all the nitrogen is excreted as uric acid, which is produced in the liver by the synthesis of urea and carbon rich residues. To a small extent this synthesis also occurs in man, but it is a slow and continuous process at present little understood. The great part of the endogenous purine excretion in man is therefore derived from the oxidation of purines in the cell nuclei, etc.

The/

The Influence of Drugs, Disease, and Physiological Conditions upon the Endogenous Purine Excretion.

- (1) Exercise causes a definite increase.
- (2) Fever causes a definite rise in uric acid excretion owing to the increased combustion processes occurring in the tissues.
- (3) Leukaemia causes a rise owing to the disintegration of the nuclei of the abnormal cells.
- (4) Gout. No very abnormal anomalies of excretion have been brought to light except perhaps that after the ingestion of purine rich food stuffs it takes longer for the resulting exogenous excretion to develop and to pass away.
- (5) Salicylic acid causes a rise by facilitating the excretion of the uric acid, rather than by increasing its formation. Caffeine causes a slight but progressive increase lasting for about ten days after its administration has been discontinued.

The Uric Acid of the Blood.

The estimation of the blood uric acid content may be accurately determined by Folin's Method, the normal is taken to be 1 to 3mg.%. The interpretation of the results is somewhat difficult as two definite and distinct processes are responsible for the maintenance of/



of the blood uric acid titre - uric acid production by the organs, and its excretion by the kidneys.

#### Uricaemia in Gout and Nephritis.

In typical cases of gout the uric acid of the blood rises from the normal to about 10mg.%, this degree of uricaemia cannot be held responsible for the deposition of urates in the joints, etc., as the blood is capable of dissolving much larger quantities of uric acid than are ever present in gout. The real cause for the deposits must depend upon some change affecting the blood so as to alter the form in which uric acid exists therein, with the result that it passes into the joints and is deposited there.

In lead poisoning and nephritis a definite uricaemia is present, in the latter it is due to retention by the kidneys.

Salicylates and atophan diminish the blood uric acid content at first, but it gradually reaches its old level even whilst the administration of the drugs is being continued.

The above account is abstracted from Physiology and Biochemistry in Modern Medicine - MacLeod (1922).

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### CLINICAL APPLICATIONS.

#### Aromatic Toxin Conjugation.

Foster and Kahn (1) in a paper in 1916 discussed the whole subject of liver function tests, and after a cursory review of the functions of the liver and the various tests that have been used to investigate them they describe a method based upon the conjugation of certain aromatic toxins - sulpho-conjugation. The main points in their paper may be summarised as follows:-

Phenol, cresol, skatol, indol, etc., are formed in the intestine by the action of bacteria upon the amino-acids that have been produced by the digestive ferments. These bodies are absorbed and are toxic to the organism until they have been conjugated with sulphuric acid and glycuronic acid. The consensus of opinion is that this conjugation takes place in the liver. To give a definite example - the amino acid tryptophane is broken down in the intestine, and one of the products of its decomposition is indol. In the intestine the indol is oxidised to indoxyl before absorption. In the liver the toxic indoxyl is conjugated with sulphuric acid to form the non-toxic indican or indoxyl sulphate of potassium, and as such it is excreted by the kidneys. For each of the other aromatic toxins a similar conjugation takes place, either/

either with sulphuric acid or with glycuronic acid, but it is only with the former that we are concerned here. In the urine these products are excreted as the ethereal sulphates.

The total sulphur of the urine is composed of (1) Inorganic S. (2) Ethereal sulphates and (3) Neutral S. The inorganic sulphur is 70% of the whole and the other two have the remaining 30% equally divided between them.

If the liver is insufficient its power of conjugating the toxins may be diminished and consequently the ethereal sulphates in the urine would be reduced in amount. In calculating this amount it is essential to take into account the quantity ingested, and the nature of the intestinal flora.

#### Technique.

The patients were put on a standard diet after the bowels had been well cleared with a dose of castor oil, and the total sulphur and the ethereal sulphates were estimated daily. On the third day the patient received a capsule containing 0.5gm. of thymol; the urine was collected for the next two days for the purpose of estimating the total sulphur and the ethereal sulphates. If all the thymol were absorbed, and all conjugated with sulphuric acid and none with glycuronic acid, the 0.5gm. would be excreted as 0.766 gm. of thymol sulphuric acid. This would cause a/

a marked increase in the percentage of ethereal sulphates. If the liver were not functioning properly the thymol would not be fully conjugated and the percentage of ethereal sulphates would be only moderately increased or perhaps not at all.

In twelve cases the findings were recorded, in the non-hepatic cases and cases of non-destructive diseases of the liver a marked increase in the excretion of the ethereal sulphates was noted on the day following the ingestion of thymol; in actual hepatic cases, such as cirrhosis, cancer, and syphilis, the liver had lost its power to conjugate and little increase occurred.

In a critical review in 1920 Labbé and Bith (2) discuss the tests of liver function that had been used up to that time, their paper is practically a résumé of the work of the French school and it is as complete as possible. Most of their original work was done upon the relation of the liver to protein metabolism and it will be well to give a full description of their views.

#### The Proteolytic Function of the Liver.

Amino-acids that are brought to the liver are split there into two portions, the one is the nitrogenous portion and is excreted as urea and ammonia, the other is the carboxyl portion and goes to the tissues to supply their needs. Disturbances in the proteolytic/

proteolytic function of the liver may be studied by the changes in the urine and the blood.

### THE URINE.

#### (1) Diminished amount of urea in the urine.

The normal amount of urea excreted in 24 hours is 25-30 grammes, and if it falls below 15-20 grammes per diem the diminution is considered to be present, the only conclusion that can be drawn from the presence of this condition is that the nitrogen intake has been diminished.

#### (2) The Urea coefficient.

This is the relation of the urea nitrogen to the total nitrogen,  $\frac{\text{Urea nitrogen}}{\text{Total nitrogen}} = 80-86\%$  normally, but there are physiological variations according to the diet, thus the coefficient is higher on a meat than on a vegetarian diet. In disturbances of liver function this ratio may fall and it has been seen to drop as low as 44%; usually even in severe cases it is between 70 and 80%. The reaction is not of great practical value as the technical difficulties militate against the accuracy of the results.

#### (3) Ammonia.

The daily excretion of ammonia is 0.40 - 0.70 gramme, and in the course of liver diseases this rises/

rises at the expense of the urea. The ammonia coefficient  $\frac{\text{Ammonia nitrogen}}{\text{Total nitrogen}} = 2-5\%$ , and rises as stated above in liver diseases and in acidosis.

(4) The Coefficient or "Ureogenic Imperfection."

This is represented by the ratio:-

$$\frac{\text{Ammonia nitrogen}}{\text{Am. nit.} + \text{urea nit.}} = 4.5-7\%.$$

This is a further modification of this coefficient, the Amino-acid nitrogen being added to both top and bottom lines. This is considered an excellent method of estimating proteolytic insufficiency of the liver but the presence of acidosis renders it valueless.

(5) Provoked "Ammonuria".

By giving ammonium salts to patients upon a standard diet it is possible to estimate the increase of ammonia in the urine if the liver is unable to cope with it. In about 50% of hepatic cases the test is positive.

(6) Amino-Acids.

The daily excretion of the amino-acids is 0.05-0.25cg., and this amount becomes greatly augmented in fatty degeneration of the liver, in infections and intoxications (e.g. chloroform and phosphorus) primary cancer, etc. In certain cases the escape of the acids may be "selective", that is to say, only a few of/



of them may appear in the urine - cystine, leucine and tyrosine, and more rarely alkapton.

(7) Ratio of the Amino-acids to the Total Nitrogen.

This is the so-called coefficient of amino acidolytic insufficiency. 
$$\frac{\text{Amino Acid nitrogen}}{\text{Total nitrogen}} = 0.5-2.5\%$$

and the raising of this ratio is a most important index of liver disfunction, it may rise to 12%; rarely it may rise in cases of very rapid emaciation. In diabetes mellitus it reaches its highest level in the neighbourhood of 20%.

(8) Provoked Aminoaciduria.

By giving glycocoll, alantine, aspartic acid, or more simply, a commercial peptone (Labbe) which contains polypeptides and amino acids, and dosing the amino-acids in the urine before, after and during the experiment it is possible to provoke a aminoaciduria in cases which did not show it before the administration of the amino-acids. It is thus possible to expose a latent inability of the liver to deal with the amino-acids and a test of some delicacy is available.

(9) Colloidal Nitrogen.

This belongs to the incompleated oxidised protein derivatives, and any increase in the colloidal nitrogen, total nitrogen ratio above the normal of 0.25-1.45% is/

is significant of liver disorder in the absence of diabetes. The colloidal nitrogen is very difficult to estimate accurately so that the practical applications of the tests are limited.

(10) The Uric Acid and Purine Bases.

The site of the transformation of the purine bases into their end product uric acid is not definitely decided, and it is impossible to impute disturbances in the uric acid metabolism to liver disease or functional insufficiency.

(11) Creatine and Creatinine.

It is known that in liver disorders and diabetes the excretion of these substances is increased.

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THE BLOOD SERUM.(1) The Residual Nitrogen.

The residual Nitrogen is composed of the difference between the total and the urea nitrogen in serum that has been freed from albumen, normally the figure is 10cg.%. Any increase is related to insufficiency in the proteolytic function of the liver, as in advanced cirrhosis, abscess and cancer of the liver, chronic venous congestion (cardiac) acute infections, etc. This is the generally accepted view and Labbé and Bith subscribe to it, but they did not find the increase proportional to the amount of liver damage, as it was highest in cases of diabetes (52cg.%) in which no anatomical lesions could be demonstrated at the autopsy. The gravest objection to the procedure is the extreme delicacy of the chemical technique.

(2) Amino Acids.

Using ordinary blood serum from which the albumen had not been removed the amino acid content was found to be 0.01-0.05cg.%, in liver insufficiency and diabetes mellitus this figure rose.

(3) Acidosis.

The authors regard acidosis as due in many cases to liver insufficiency in the absence of diabetes.

(4)/

#### (4) Sulphur Function.

The metabolism of sulphur is closely related to the proteolytic function of the liver, as deficient oxidation of the sulphur belonging to certain molecules is a sign of another form of incomplete amino-acidolysis, the estimation of the neutral sulphur elimination of the 24 hours is therefore of some interest.

#### Neutral Sulphur.

Normally the sulphur of the protein molecule is modified in the liver in three different ways.

- (1) By oxidation and combination in the form of sulphuric acid with bases it forms sulphates and is excreted as such.
- (2) By conjugation with certain aromatic toxins such as indol, phenol, skatol, etc., to form the ethereal sulphates.
- (3) A very small quantity is not acted upon by the liver and it passes through this organ unchanged and it is excreted as cystine, oxyproteic acid, or taurine - this is the neutral sulphur, and normally it is about 10-15% of the total sulphur.

If the liver is insufficient the neutral sulphur percentage will rise and it may even reach the figure of 35% or more, if such large quantities are excreted the conditions of cystinuria is caused. This test is of some interest but the difficulties in the way of making an accurate estimation militate against its acceptance in the clinical work.

### CONCLUSIONS.

The authors regard the study of nitrogenous metabolism as of the first importance in the assessment of liver damage, and it is only in conditions of very massive tissue destruction as in traumatism, infections and rapid carcinomatosis that the extra-hepatic proteolytic processes can acquire a great importance. The rôle of the liver on the other hand is to a greater or less extent usurped by other tissues and this fact makes a study of these functions rather more difficult to estimate. The urine is the seat of election for the various tests as the blood serum presents too many difficulties to the bio-chemist to allow of frequent and rapid estimations to be made.

Kinberg (3) reviews the literature and reports the results of much personal research upon the elimination of amino-acids and ammonia in healthy subjects and those who were the victims of liver disease. He tested the functional capacity further by the ingestion of gelatine instead of glycocoll, after a constant diet with a low nitrogen content for several days 50 grammes of gelatine dissolved in hot chocolate were taken fasting. The metabolic findings for 14 liver cases and 6 healthy persons are tabulated. He did not find any increase in the amino-acid output with liver/



liver except after the gelatine test, then the output increased but only in serious pathological conditions of the liver, such as advanced cirrhosis, etc., but no change was noted in transitory disorders like catarrhal jaundice or congestion. The output of ammonia was almost always higher in liver diseases than in health, both absolutely and relatively.

Pezzali (4) in an extensive research into the various methods of testing the functional capacity of the liver came to the conclusion among other things that the estimations of the urea and ammonia content of the blood and urine do not furnish reliable data; he endeavoured to determine experimentally the metabolism of nitrogen in the blood above and below the portal system in various conditions of feeding. He was, however, unable to discover any technique that would enable this differentiation.

Labbe, Hutinel, and Nepveux (5) take the view that in hyperemesis gravidarum the acidosis and diaceturia, etc., are due to liver insufficiency and not to fasting and carbohydrate privation. In support of this contention they quote a fatal case of this disease which showed the usual signs of proteolytic insufficiency of the liver with the autopsy findings of central necrosis of the lobules of that organ.

Delprat and Whipple (6) suggest that the synthesis of hippuric acid might be made use of as a test/

test of liver function - this conjugation of benzoic acid and glycocoll to form hippuric acid has been held to take place in the kidney, but recent evidence has rather proved that it is to some extent a liver function. Details for the reason of this view are given in the paper. The authors found experimentally that whilst normally the administration of benzoic acid causes an increased excretion of hippuric acid, if a chloroform liver necrosis is produced the synthesis of this acid still takes place, but it is delayed. They suggest that this means that the liver normally takes part in the synthesis, but in an emergency the cell protoplasm of the other organs may be called upon to take over the greater part of the hippuric acid synthesis. The paper is of physiological interest but as yet there have been no practical outcomes of the work.

A series of three papers on the estimation of phenols in the blood appeared in the year 1922. The first by Pelkan (7) gives the method which he used; he argues that the phenols formed in the alimentary tract by the bacterial decomposition of protein are practically all excreted in the urine in a conjugated form - as there is no evidence to show that they are formed in the kidneys or bladder and as their formation is admittedly a function of the liver it was assumed that they must pass through the blood stream on their way/

way to the kidneys for excretion.

#### Method for the Estimation of the Blood Phenols.

10ccs. of blood are added to 50ccs. of distilled water in 100ccs. flask, then 10ccs. of a 10% solution of sodium tungstate and 10ccs. of a two-thirds normal solution of sulphuric acid are added, the flask is stoppered, and shaken. To precipitate the proteins completely 10ccs. of aluminium cream are added and shaken. Transfer to a 100ccs. centrifuge tube and centrifugalise for 45 minutes. The supernatant fluid is filtered to the 45cc. mark in a 50cc. graduated flask, 5ccs. of a 5% solution of lactic acid are added and the graduate is shaken for one minute. After further centrifugalisation and filtration the filtrate is ready to be examined for phenols.

Two test-tubes are required, one graduated to 15ccs. and the other to 10ccs.

#### The Free Phenols.

The 15cc. tube is filled to the mark with the filtrate, and 1cc. of the phenol reagent<sup>\*\*\*</sup> is added, and the tube is shaken. The excess of silver precipitates out, and the solution is filtered to the mark in the 10cc. tube, and 5ccs. of a 20% solution of sodium carbonate are added. This solution is now transferred to another tube, and the colour develops to a maximum in 20 minutes.

#### The Total Phenols.

The/

The 15cc. tube is filled to the mark with some of the filtrate, 5 drops of concentrated hydrochloric acid are added, and the tube is placed on a water bath at 100° for 10 minutes, then 1cc. of the phenol reagent is added and the estimates continued as for the free phenols.

#### The Standard Solution.

5ccs. of a stock solution of resorcinol (Benedict and Theis - Journ. of Biol. Chem. 1918: 36: page 95) containing 5.81mg. are placed in a 100cc. volumetric flask, 0.5cc. of concentrated hydrochloric acid and 10ccs. of the silver lactate-lacticacid solution are added, the mixture is centrifugalized, and the filtrate is manipulated in a graduated flask as in the estimation of the blood phenols.

The estimation is performed in a Dubosc colorimeter against the standard.

#### \*\*\* THE PHENOL REAGENT.

100gms. of sodium tungstate, 20 gms. of phosphomolybdic acid, 50ccs. of an 85% solution of phosphoric acid, and 100ccs. of Hydrochloric acid, is gently refluxed for two hours with 750ccs. of water, and at the end of that period of heating it is made up to 1000ccs. with water.

The next paper by Pelkan and Whipple (8) is devoted/



devoted to the physiology of the phenols.

A small part of the tyrosine of the food proteins is broken down in the intestine by bacterial action into hydroxy acids such as p-oxyphenyl propionic acid, p-oxyphenylacetic acid, and p-oxybenzoic acid; the remainder into volatile phenols, primarily p-cresol, and phenols. The hydroxy acids have no toxic effects and they are not subjected to any noticeable degree to oxidation or conjugation. They are almost completely excreted in the urine as free phenols. The volatile phenols are dealt with in an entirely different way as they are very toxic even in small quantities. More than half of them is oxidised by the intestinal mucosa, the body fluids and the liver parenchyma. The remainder is conjugated in the liver with sulphuric and glycuronic acids. After passing from the liver the conjugated phenols are uniformly distributed in all the tissues and are rapidly excreted by the kidneys in 12 hours. From their experimental work the authors feel confident that the synthesis of phenolsulphuric acid and of phenolglycuronic acid takes place only in the liver parenchyma.

The third paper is devoted to experimental work upon animals. The following are the conclusions arrived at:-

- (1) The presence of a slight liver injury produced by chloroform or phosphorus may not modify the phenol/



phenol conjugation, extensive liver injury due to these poisons will always lessen the conjugation, and extreme or fatal degrees of liver injury will reduce the conjugation to zero.

- (2) The presence of an Eck fistula modifies the conjugation of the phenols and reduces its amount and speed. In time the Eck fistula liver function of conjugation may fall to  $1/3$  or even  $1/10$  of the normal. When the liver circulation is still further impaired by partial ligation of the hepatic artery the conjugation of phenol may fall to 3% or 5% of the normal.

Liver exclusion will therefore eliminate phenol conjugation.

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CRITICISM OF THE TESTS.

It is an undoubted fact that the liver plays an important part in protein metabolism, and that in serious liver disorders of that organ there is a disturbance of this function, but, although a very large amount of work has been done upon the subject, the results of the investigations of the nitrogenous output in liver cases have been disappointing. The tissues are able to take over, to a certain extent, this function, and it is not possible to discriminate in a given case the extent to which this usurpation has gone.

The so-called nitrogen partition tests depend for their interpretation upon a study of the nitrogen content of both the excreta and the ingesta before accurate conclusions can be drawn, as the study of the excreta alone does not take into account the various modifications that a change of diet can produce; it is necessary therefore to investigate the nitrogen balance and this complicates the performance of the tests. As the relations between the different bodies which are estimated in these tests depend not only upon a disordered liver function, but to an equal, if not greater, extent upon acidosis and diabetes, and other profound metabolic upsets they cannot be regarded simply as tests of liver function.

The paper of Labbé and Bith contains a very fair summary/

summary of the subject, and it will be seen that in practically every test which they mention as being affected by liver diseases, acidosis and diabetes mellitus can produce a similar effect, and usually to a much greater degree.

An additional objection to the application of the nitrogen partition tests in the study of liver disorder is that the chemical manipulations render the services of a biochemical laboratory essential, and even with this facility the results are usually not commensurate with the expenditure of time and energy that is entailed.

Turning to the tests that depend upon the conjugation and detoxication of the toxic end products of the putrefaction of the ingested proteins in the alimentary tract, the simplest one is the test of glycuronuria, the value of which is discussed in another section. Several papers have been quoted on the subject of the excretion of ethereal sulphates, and on the conjugation of phenol, but sufficient work has not been done yet to allow of any definite conclusions being drawn; the elaborate technique required would seem to be a grave objection to their use as a routine measure in the clinique.

The excretion of leucine and tyrosine - a form of aminoaciduria - is an indication of active destruction of the liver cells.

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THE LEVULOSE TOLERANCE TEST.



The evolution of the levulose tolerance test in its present form has been a slow process, intimately connected with the growth of knowledge of that particular hepatic function which it is used to test, the glycogenic function.

It dates back, as does so much of modern physiology, to an experiment of Claude Bernard's in which progressive ligation of the portal vein was found to produce a spontaneous glycosuria with a marked augmentation on the ingestion of sugar. Colrat (1) and Lepine in 1876 were the first to apply ~~for~~ these facts in the realm of clinical medicine, but they regarded spontaneous or provoked glycosuria as indicative of partial obstruction of the portal vein; Colrat's test (the ingestion of 150-200 grammes of sugar solution with the examination of the urine for sugar during the succeeding twenty-four hours) came to be regarded as the best proof of hepatic derangement; when the inversion of saccharose by the pancreatic ferments was shown to occur glucose was introduced to simplify the test.

Achard and Castaigne (2) in 1899, Linossier (3) in 1899, and Castaigne (4) in 1910 condemned the test as erroneous unless special precautions were taken to prove the absence of spontaneous glycosuria and to prove that the renal function was normal by using the methylene blue test, and if glycosuria was provoked they/

they inferred liver insufficiency. Linossier (5) in a further paper showed that cases of cirrhosis were often positive, but that normal persons also were frequently so.

Strauss (6&7) and Sachs (8) were the first to introduce the test of what they called alimentary levulosuria, using 100 grammes of levulose as the maximum amount usually tolerated in health. Their results were confirmed by numerous workers, amongst them may be mentioned Ferranini (9), Halasz (10), Bruning (11), but Landsberg (12) and Churchman (13) found that the inconstancy of the test rendered it unreliable.

Parenthetically it may be mentioned that provoked galactosuria was the subject of several papers, (14, 15, 16, 17); it was shown to be of value only in cases of infectious jaundice, and to be thus of very limited applicability.

Brun (18) advocated the use of adrenaline glycosuria.

Achard and Desbouis (19) in a series of papers review the subject of the testing of liver function by the production of glycosuria and, more important to the matter in hand, levulosuria, and they concluded that the tests depended upon normal absorption, normal renal threshold, and a constant individual tolerance, and it is only after these have been proved to exist that/

that the question of sugar fixation as proving normal hepatic function can be mooted. They are of opinion that sugar fixation is less a test of liver function than of tissue utilisation which depends chiefly upon endocrine balance.

Maclean and de Wesselow (20) showed the inaccuracy of measuring sugar tolerance in general by estimating the elimination of glucose by the kidneys; in a most able paper they discuss at length the whole subject, and a short resumé of their views and findings is of some importance in explaining the reasons for the adoption of the levulose tolerance test in its present form.

Following the ingestion by the mouth of a sugar such as glucose, and studying the changes which result by half-hourly estimations of the blood sugar percentage, and of the urinary sugar it was found that the results - in a normal subject - varied with the dosage of the sugar, but a relatively enormous quantity had to be given (up to 400 grammes) in many cases before glycosuria was produced. After a moderate amount of glucose - 50-100 grs. a very typical curve of blood sugar content resulted:-

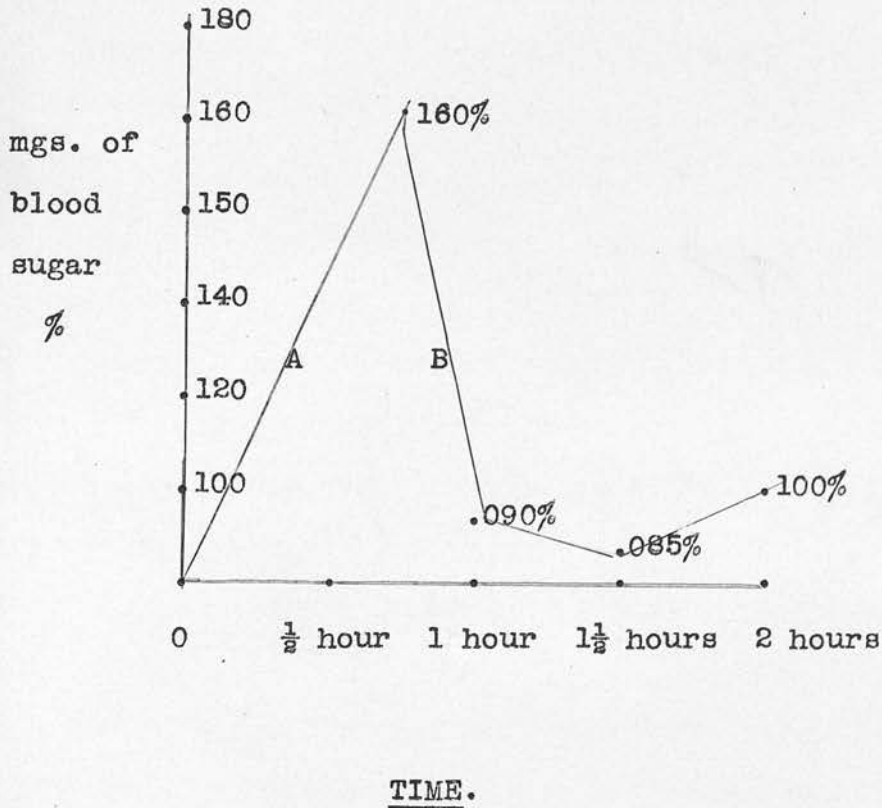


Figure 1.

Normal blood sugar curve after 50grs. of glucose. The blood sugar content rises rapidly during the first half-hour to a maximum of .160% or thereabouts, and falls subsequently to normal or slightly below it at the end of an hour. The renal threshold for glucose is about .180%, and unless the blood sugar content surpasses this amount no glycosuria will occur.

The authors regarded the curve as being formed of two separate parts - A the rise and B the decline; the rise they regard as being due directly to the absorption into the blood stream of the sugar from the/

the intestine, and the decline as due to the storage of the sugar by the liver prior to its utilisation. In a long series of experiments they were able to satisfy themselves that A did not vary materially from alimentary causes, and that the error of introducing the sugar into the body through a membrane of varying degrees of absorptive power could be neglected.

The main application of this method of estimating the sugar tolerance was, of course, to diabetes mellitus, where a prolonged rise and a slow fall are obtained; from the present point of view several facts of capital importance emerged, and these remain to be dealt with.

The fallacy of the so-called provoked glycosuria was shown to be the great variation of the renal threshold in both abnormal and normal states - in diabetes mellitus and chronic nephritis the threshold may be as high as 0.25%. For this reason Strauss's test was open to grave objections.

In studying the curves following the ingestion of different kinds of sugars Maclean and de Wesselow found that normally levulose in doses of 30-50 grammes produced no alteration in the blood sugar content, and the explanation they offer is that the liver is able to store this amount with the same celerity as the alimentary canal absorbs it. Hence the levulose tolerance test of the present day.

In these experiments the blood sugar was estimated/



estimated by Maclean's now well-known micro-chemical method, in which the quantity of blood necessary (0.2cc.) may be obtained by means of an ordinary prick thus obviating the unpleasantness of repeated venipuncture. For details of the method reference should be made to Maclean's small monograph (21).

Spence and Brett (22) were not tardy in applying the test to the elucidation of cases of salvarsan jaundice furnished them by Harrison, and it was upon their paper that the present <sup>writer</sup> founded his own work, a brief review of their technique and findings must therefore be included.

After a period of at least five hours' fasting a blood specimen was taken, and then the levulose was administered in 100-200ccs. of water, the dose being regulated according to the weight of the patient, thus:-

A patient weighing 80 kilos. received 50 gms. of levulose.

"	"	"	60	"	"	40	"	"	"
"	"	"	40	"	"	30	"	"	"

At subsequent intervals of half an hour specimens of blood were withdrawn until the end of the second hour, and the five specimens were then examined by Maclean's method for the sugar content.

The/

The following are three of their curves:-

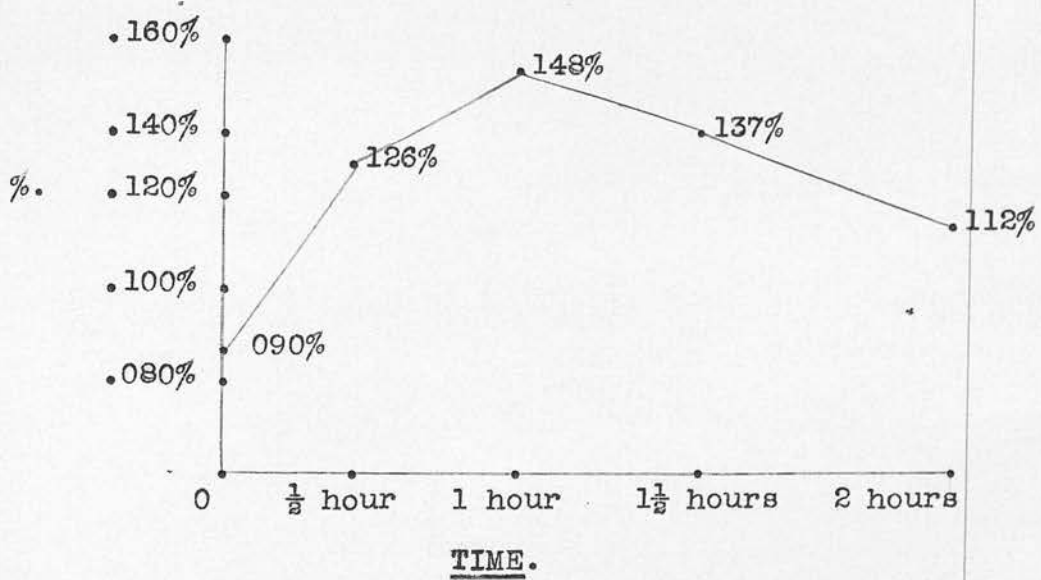


Figure 2. Levulose tolerance test. Jaundice - clinically moderate.

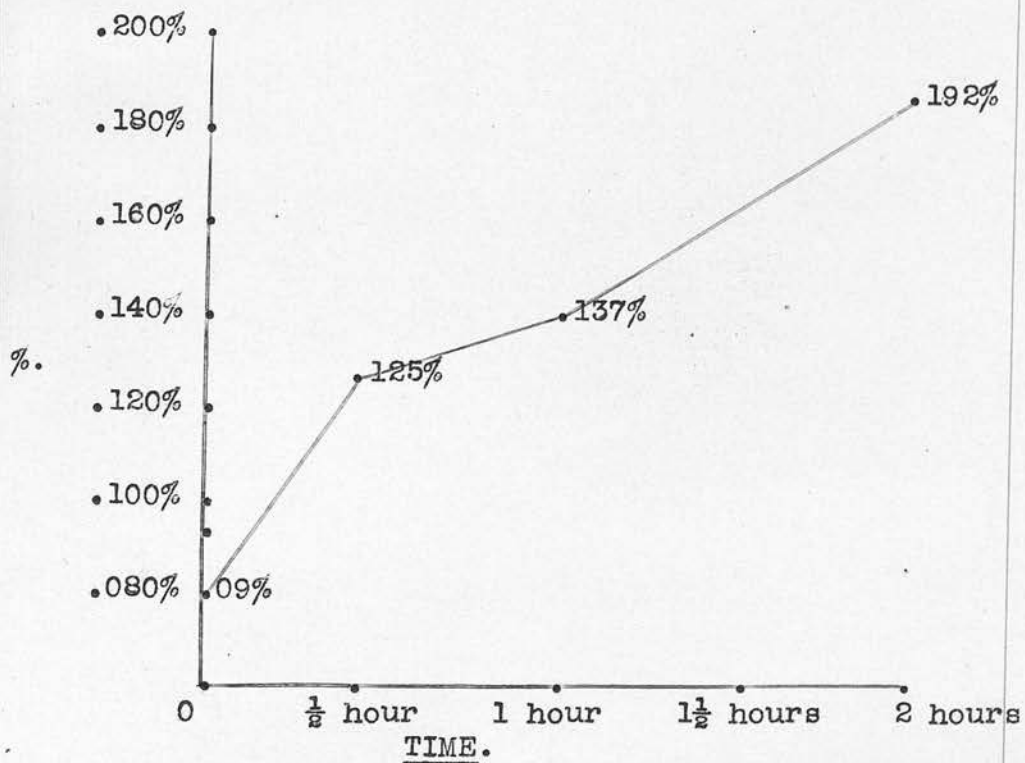


Figure 3. Levulose tolerance test. Jaundice - clinically severe.

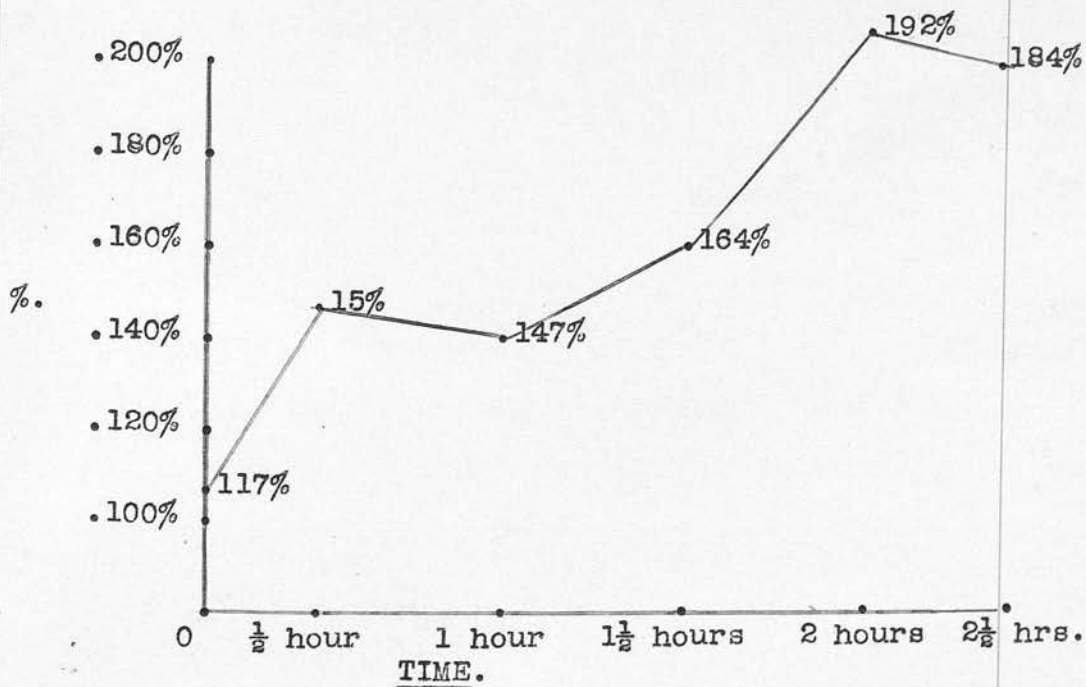


Figure 4. Levulose tolerance test. Jaundice - Fatal.

They regarded a rise of 0.02% as being abnormal, and direct evidence of the failure of the glycogenic function of the liver, and they claimed that a prognosis could be given in these cases which was much more accurate than that of the clinician on clinical evidence alone.

Covell (23) applied the method to the study of tropical diseases, and he claims to be <sup>the</sup> first to have done so. He adopted the technique of Spence and Brett, with the exception of the blood sugar estimations for which he used the method of Folin and Wu (24) the blood being withdrawn from a vein. He divides his cases into four groups which will be dealt with in the order which he gives.

(a) Normal Students.    10 cases.

These showed a maximum rise of 0.012% at the end of one hour.

(b) General Medical Cases.    13 cases.

Nine out of thirteen cases showed no abnormality in the curves, and clinically none was to be anticipated. 4 cases showed a definite rise in blood sugar beyond the limit of normality; these were chronic bronchitis and alcoholism (0.028% rise at the end of one hour), tuberculous peritonitis (0.018% rise, - doubtful case), epilepsy and arteriosclerosis (0.022% rise, arsenical peripheral neuritis (0.040% rise - next day 0.030%).

(c) Clinical Hepatic Disease.    14 Cases.

The test gave apparently reliable results in various conditions, malignant disease of the liver, Banti's disease, silver salvarsan poisoning, etc. One case of obstructive jaundice gave a negative result (cancer of the head of the pancreas with a liver reaching to the umbilicus), and one case of doubtful cirrhosis of the liver also was negative.

(d) Tropical Diseases.(1) Sprue.    6 cases.

3 cases gave a normal curve, one a moderate degree of insufficiency (0.031% rise after one hour), and 2 cases showed slight degrees.)

(2) Amoebiasis.    15 cases.

8 cases of apparently cured dysentery gave normal curves. The remainder of the cases were either chronic dysentery or liver abscesses that had been operated upon, and they all showed some abnormality in the curves. Unfortunately the author was not able to obtain a case of acute amoebiasis or an abscess before surgical intervention.

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CONCLUSIONS.

- (a) He confirms the value of the test and the work of Maclean, Spence and Brett.
- (b) The test is a valuable indication of hepatic disease in certain cases where there is no clinical evidence of it.
- (c) That in chronic sprue there was little evidence of hepatic disease.
- (d) That in amoebiasis the liver is affected to a greater or less extent in the majority if not in all cases.

Gerrard (25) in a paper quoted at length elsewhere found the test too time-consuming for ordinary work, and he was forced to abandon it on this account.

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Horak (26) studied the hyperglycaemia following the injection of levulose and the excretion of indigo-carmin by the liver in tuberculous animals. Rabbits showed no changes even with advanced tuberculosis of the lungs. Guinea-pigs with affected livers showed an abnormal sugar curve.

Bloomfield and Hurwitz (27) in a critical review in 1913 of the tests of liver function depending upon the use of carbohydrates came to the conclusion that the tests were of no value. To quote from their paper "A consideration of the extrahepatic factors involved in the sugar regulating mechanism, the influence of the endocrines, the ability of other tissues than the liver to handle sugar, the action of the autonomic nervous system, and the ability of the uninjured liver substance to compensate in disease makes the sugar tests theoretically unsatisfactory. There are a series of great practical difficulties in applying the tests, nausea, vomiting and diarrhoea after feeding, faulty absorption, intestinal fermentation, portal obstruction with a collateral circulation, retention of sugars in nephritis, etc. There are serious objections to the methods as they have been applied, namely, the use of arbitrary amounts of sugar, and the use of a definite standard of excretion.

An analysis of the reports in the literature shows/

shows their significance to be lessened owing to confusion in the conception of hepatic insufficiency, insufficient clinical data, and the neglect of the practical considerations mentioned above".

Duzar and Hensch (28) found with the alimentary hyperglycaemia test (glucose), in healthy, breast-fed infants, from 6-10 weeks old, a blood sugar content of 0.15 mg.%. The maximum rise of the curve occurred usually after 40 minutes. The levulose test was negative. The cases of congenital syphilis showed a disturbed liver function in 100% with the levulose test, in 72% with the alimentary hyperglycaemia test. The tests showed relative insufficiency of the liver in pyelitis, grave anaemia, icterus neonatorum, and in Leiner's dermatitis.

Jacobson (29) in an experimental research into the effect of an Eck fistula upon the sugar tolerance of animals came to the following conclusions.

- (1) Eck fistula animals have an extremely low tolerance for levulose. The liver is evidently essential for levulose metabolism. The function of converting levulose into glucose is possessed by the liver. This function is permanently lost when the portal blood is diverted into the vena cava.

(2)/

- (2) Glucose tolerance is only slightly modified in Eck fistula animals. The liver is not essential for glucose metabolism. The muscles undoubtedly perform well the functions of glycogenesis and glycolysis when the liver is shunted out of the portal circulation.

#### CONCLUSIONS.

- (1) That the levulose tolerance test is dependent upon the functional capacity of the liver is proved by both clinical and experimental work.
- (2) That the test has several grave objections, it is very time-consuming, and the production of nausea and vomiting is an occurrence which renders the applicability of the test very limited, as most patients with a liver lesion of any severity are very prone to gastric upsets.
- (3) That the test is one of the best there is at present for testing liver function.
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THE SODIUM SALICYLATE TEST.

In a comprehensive paper in 1922 Roch of Geneva (1) gives a review of many of the liver efficiency tests, and criticizes them on the grounds that each test seeks to <sup>prove</sup> the sufficiency of only one function of the liver and this fact is frequently overlooked that in the event of some hepatic disorder the various organs of the body are able to act in some degree as substitutes. For instance, the glycogenic function may be aided by the muscles and the pancreas; the spleen is able to assist in haemolysis; the supra-renal capsules in the formation of cholesterolin; and the kidneys in the mechanism of antitoxic defence.

Many of the tests require a very intricate technique with elaborate laboratory methods, and they may even put the patient to a considerable degree of physical discomfort.

A final objection which he mentions is that many of the tests are depended upon - not liver function - but also the vagaries of alimentary absorption and renal efficiency; accordingly Roch suggests the sodium salicylate test, as this body is easily diffusible, and not much prone to variations on account of abnormal alimentary conditions.

The test was actually heralded by Labbé and Bith (2), but it was first seriously applied by Roch (3) and the method was standardised later by Roch and Schiff/

Schiff (4). The test depends upon the fact that normally 4 centigrammes of salicylate of sodium ingested by a fasting patient are completely fixed by the liver for the glycuronic acid conjugation, and its passage into the urine is thus prevented.

Much of the clinical work was done by Dimietriyevitch and incorporated in his thesis for the degree of M.D. at the University of Geneva. By the courtesy of the Secretary of this University the writer has been enabled to borrow a copy and a summary of it will be given in this section.

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SUMMARY OF THE THESIS OF DIMIETRIYEVITCH. (5).

After an introductory chapter upon liver efficiency tests the author proceeds to a description of the technique of the sodium salicylate test:-

Method of Administration.

At 7 a.m. the patient is given the ordinary hospital breakfast of a cup of coffee and milk and a roll. The urine from 7-8 a.m. is collected and used as a control.

At 8 a.m. he received a pastille of 0.04 grammes of sodium salicylate. The urine from 8-9 a.m. is discarded, but the two specimens collected between the hours of 9-11 a.m. and 11 a.m. - 1 p.m. are kept for testing.

The reasons for these steps may briefly be stated; a solution of sodium salicylate was found to give the same results as the tablets, but the tablets were more convenient as they obviated the measurement of such small amounts of the salt. After numerous experiments it was found that the normal threshold for salicylate was 0.037-0.038 grammes, and 0.04 gramme was chosen as the most convenient dose. The previous administration of a meal is for the purpose of stimulating the liver to full activity before estimating its functional capacity.

7-8 a.m. urine. This is the control, and it is used/



used to ascertain whether the urine has any effect upon the solution of ferric perchloride which is employed to detect the presence of the salicylate; the bodies that may give a reaction with the iron are diacetic acid and certain medicaments of the aromatic group.

8-9 a.m. urine. This is discarded as it was never found to contain salicylate except in cases of exceptionally quick transit after very heavy doses when it may appear in 15-30 minutes (Chelchowski (6).)

9-11 a.m. and 11 a.m. to 1 p.m. urines. On these the actual tests are performed; it was found at the outset that a test was never positive in a third specimen taken from 1-3 p.m. so that it was possible to suppress this specimen. In 80% of the cases the first glass alone sufficed to gauge the reaction, and in 20% of the cases the second alone was necessary. The author is at present experimenting upon the practicability of using a single specimen from 10 a.m. to mid-day.

#### The Method of Testing.

Into a test-tube containing an aqueous solution of ferric chloride similar in depth of colour to the urine (1%-2%) the urine to be tested is dropped by means of a pipette of gross calibre delivering drops of about .3cc. A positive reaction is indicated by a violet or "violaceous" colour being assumed by the drops/

drops, a doubtful reaction by a slight violet tint, and a negative reaction by no colour developing at all. The examination should be done in reflected light using a white background ordinarily, but in doubtful cases a black background makes the interpretation somewhat easier.

It is important to have a similarity in colour between the urine and the ferric chloride solution.

#### Acidulation of the Urine.

In alkaline, amphoteric, and slightly acid urines a cloud of phosphates is precipitated by the perchloride, and the colour changes are masked; the urine must therefore be acidulated either by the addition of pure nitric acid or of pure sulphuric acid, using three drops of the former or one drop of the latter to a glass of urine so as to obtain an acidity of about 0.05% nitric acid.

#### Icteric Urines.

It is difficult to perform the test on icteric urine owing to the disparity of tint, but the urine may be strongly diluted if a test is considered to be of any value in the presence of so obvious signs of hepatic disorder.

#### Diuresis.

A very dilute urine will give a negative test, and/

and it is necessary to extract the salicylate with ether.

Dimietriyevitch then gives a full account of his seventy-six cases, sixteen of whom were normal persons, with details of various other chemical reactions in the urine, and in some cases a comparison of several methods of assessing liver function - such as Roch's Methylene Blue test, glycuronuria, the presence of bile salts and pigments. These are set out in tabular form at the end of this section.

In the final section of the thesis the author discusses the validity of his test as one of liver function, and briefly his arguments are as follows:-

Defective functioning of the alimentary tract does not appear to vitiate the test; according to Grisson (7) the normal stomach exercises no action upon the salicylate compounds, and there is no reason to imagine that in pathological conditions it does so. In the lower part of the tract dissociation might occur under the influence of bacterial fermentation, but the rapid transit of the drug renders this eventuality unlikely. The important question is whether the kidneys exercise any influence upon the test as is the case with so many of the other tests of liver function.

According to Sahli (8) the violet colour with ferric/

ferric perchloride that is obtained in urines after the administration of drugs of the salicylate group is due to two substances - salicylate acid and salicyluric acid (oxyhippuric acid) the former occurring in twice the amount of the latter (9), and it is known since the researches of Bunge and Schmiedoberg (10), confirmed by Violle (11) that it is the kidneys that synthesize hippuric acid from benzoic acid and glycol. It might be similarly inferred that salicyluric acid is transformed there also, and that only one-third of the ingested salicylic acid is conjugated in this way, the rest being excreted unchanged.

Examining these possibilities in turn:-

A. That salicylic acid only suffers in part the hippuric acid synthesis.

Admitting this hypothesis the question is to know whether the kidneys - diseased or healthy - can exercise any influence in arresting the salicylic acid. If they cannot it must be admitted that if 0.037 to 0.040 gramme of the acid is not excreted in a supposedly healthy person that the liver has stopped it.

Dimietriyevitch is satisfied that his cases of hepatic disease with healthy kidneys giving a positive result with the test prove that the kidneys do not influence salicylate excretion, and he has several cases of Bright's disease and of renal sclerosis which have shown a normal elimination.

B./

B. That salicylic acid undergoes completely the hippuric synthesis.

In this case it is assumed that Stokvis' (12) view is correct, and that the violet coloration in the urine with ferric perchloride is due entirely to salicyluric acid. In order that the synthesis may take place the salicylic acid must reach the kidney, and when the perchloride reaction does not occur it may be concluded that the salicylic acid has all been arrested by a healthy liver, as Lewinski (13) has demonstrated the small influence that renal disease has upon the salicyluric synthesis.

It may be concluded, then, that the test is one of liver efficiency and not of renal function.

Dimietriyevitch would urge the test as one of definite value, but it is not so delicate as Widal's test of the post-digestive haemoclastic crisis.

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TABLE OF CASES.POSITIVE REACTIONS.

Disease	Size of Liver	Bile Pigment	Bile Salts	Urobilinuria.
Pulmonary Tuberculosis & Cirrhosis	Small	No	Yes	Yes
Pneumonia - during course	?	No	Yes	No
Cancer of Stomach	3f.	No	No	Yes
Cancer of Stomach	2f.	?	?	?
Case treated with N.A.B.	The salicylate test was positive for four days after the injection.			
Case treated with N.A.B.			do.	
Acute Nephritis	?	?	?	?
Chronic Alcoholism	2f.	No	No	?
Malig. Obstr. Jaundice	?	No	No	2
Mild Chr. Interst. Neph.	Normal	No	No	Slight pos.
Catarrhal Jaundice on admission:	Normal	Yes	No	Slight pos.
A few days later:	1f.	No	Yes	Yes
Chr. Alcoholism moderate	2f.	No	No	No
Chr. Interst. Neph.	?	No	Yes	Yes
Mal. Obstr. Jaundice	Yes	Yes	No	Yes
Alcoh. Cirrhosis and Ascit.	Normal	No	Yes	Slight pos.
Obesity	?	?	?	?
D.T.'s and Cirrhosis	Small	?	?	?
Pulmonary T.B.	Normal	No	Yes	Yes
Chr. Alcoholism	2f.	No	No	No
Aortic Incompetence	Normal	No	No	Yes
T.B. Pleurisy and Ascites.	Normal	No	No	Slight pos.
Alcoholism	Normal	Yes	Yes	Yes
Alcoh. Cirrhosis and Ascites	2f.	No	Yes	Yes
Parench. Nephritis	2f.	?	?	?
Obesity - Subicterus				
? Gallstones	?	?	?	?

SLIGHTLY POSITIVE REACTIONS.

Disease	Size of Liver	Bile Pigment	Bile Salts	Urobilinuria
Tricuspid Incompetence	Normal	No	Yes	Yes
Spastic Diplegia	Normal	No	Yes	No
Early Cirrhosis	3f.	?	?	?
Cancer of Liver Ascites.	v. large	Yes	Yes	Slight pos.
Pulmonary T.B.	Normal	No	No	Slight pos.
Hyperchlorhydria	Normal	No	No	No
Pneumonia	Normal	No	No	No
Chr. Alcoholism D.T's.	2f.	No	Yes	Yes
Chr. Cholecystitis with ac. exacerbation.	large	Yes	Yes	Slight pos.
do.	1f.	Yes	No	Slight pos.

NEGATIVE REACTIONS.

Cancer of Stomach	Normal	No	No	No
Hyperchlorhydria	Normal	No	No	Slight pos.
Acute Rheumatism	2f. ?	No	Yes	Yes
G.P.I.	Normal	No	No	?
Dercum's Disease	?	No	No	No
Tertiary Syphilis	1f.	No	No	No
Aortic Aneurysm	1f.	No	No	Slight pos.
Pulmonary Congestion	Normal	No	No	No
Conval. Catarrhal Jaund.	Normal	No	No	No
?	Normal	No	No	No
Urticaria	Normal	No	No	No
Paranoia	Normal	No	No	Yes
Syphilitic Meningitis	Normal	No	No	No
Addison's Disease	Normal	No	No	Slight pos.
Slight Anaemia	Normal	No	No	No
Chronic Rheumatism	Normal	No	No	Yes (physiol.)
Cholelithiasis	3f.	No	No	No
After an attack of colic)	3f.	Yes	Yes	Yes
Fibroid Phthisis	Normal	No	No	No
Chronic Neph. Alcoh.	3f.	No	No	Yes (physiol.)
Cirrhosis of Liver	Normal	No	No	No
Acute Rheumatism	Normal	No	No	Yes (physiol.)
Neurasthenia	Normal	No	No	Slight pos.
? Dementia Praecox	Normal	No	No	No

The letter and figures relating to the size of the liver indicate the number of finger-breadths below the costal margin.

AVERAGE AGE = 46 years

OLDEST PATIENT = 74 years

YOUNGEST PATIENT = 19 years

There were in addition to the above series, tests carried out on sixteen normal subjects - doctors, students etc. - they all gave negative results.

Reference to the table will show that the salicylate test appears to give positive results where these might be expected, there does not seem to be a very sharp line of demarcation clinically between the positive results and the slightly positive results, however, and many of the results are rather unexpected; perhaps a fuller case record would help to clear up the difficulty, but unfortunately, this is not forthcoming in the original thesis.

It is interesting to note the comparison between the results of the salicylate test, and the presence of bile pigments, bile salts, and urobilin in the urine.

1. Positive Cases.

Bile pigments were present in 17%.

Bile salts were present in 45%

Urobilin was present in 81%.

2. Slightly Positive Cases.

Bile pigments were present in 33%.

Bile salts were present in 55%.

Urobilin was present in 66%.

3. Negative Cases.

Bile pigments were present in 0%.

Bile salts were present in 4%.

Urobilin was present in 36%.

It must be noted, however, that the test used for the detection of the bile pigments was Gmelin's - a test which is neither accurate nor delicate; the test used for the detection of the bile salts was Hay's and that used for urobilin is not stated. Accordingly the only results which can be regarded are those obtained with the flowers of sulphur. Further reference to the comparative aspect of these tests will be found under the section devoted to tests based upon the metabolism of bile salts and pigments.

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CONCLUSIONS.

The salicylate test is one of great simplicity, but it does not give anything more than a very rough qualitative index of the state of liver function, the presence of kidney disease would seem to vitiate the results, and an analysis of the cases quoted above will show the coincidence in 50% of cases of a positive salicylate test and a positive Hay's test. From this the delicacy of the test may be gauged. It seems to have been shown that if the liver is insufficient it is not able to fix a small dose of sodium salicylate, but it is also obvious that there are other conditions which are associated with the appearance of this substance in the urine, and no specificity can be admitted for the test. The present writer's own short series contained many normal cases who gave a positive test.

Roch and his colleagues have shown only one part of their thesis, and by testing an insufficient number of controls they have conjectured the negative side of the case.

The salicylate test cannot therefore be regarded as a satisfactory test of liver function.

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TESTS FOUNDED ON GLYCURONURIA.

In France numerous researches took place from 1915 onwards into the significance of glycuronic acid in the urine, and its variation in pathological conditions; these researches purported to throw light on the anti-toxic function of the liver.

Glycuronic acid was first studied by Musculus and von Mehring in 1875, and the researches were continued by Jaffe, Schmiedeberg, Meyer, Neuberg, and Paul Meyer; in France Lépine and Boulud studied its occurrence in normal and diabetic blood, and Tiffeneau and Fredoux its conjugation with chloralose.

Roger (1) gives the following account of its chemistry:-

Glycuronic acid is a derivative of glucose differing from it by the substitution of an acid COOH group for an alcohol radicle CH<sub>2</sub>OH -

thus COH- (COH<sub>2</sub>)<sub>4</sub>-CH<sub>2</sub>OH is glucose  
whilst COH-(CHOH)<sub>4</sub>-COOH is glycuronic acid.

This appears to be a simple oxidation but in reality it is not as glucose contains an aldehyde group (COH) which oxidizes before the alcohol and gives by repeated oxidation:-

COHO-(CHOH)<sub>4</sub>-CH<sub>2</sub>H Glyconic acid.  
COHO-(CHOH)<sub>4</sub>-COOH Saccharic acid.

It/

It will thus be seen that to form glycuronic acid something more than simple oxidation must take place, as it is first necessary to conjugate and block the aldehyde radicle before the alcohol radicle can be oxidized; many substances can do this but they are all of the oxhydrile, alcohol or phenol groups, and they will be dealt with later.

There are many methods for the chemical detection of the acid in urine, but two only will be given.

A. Method of Tollen and Rorive, modified by Grimbert and Bernier.

20ccs. of urine are shaken up with 10ccs. of cold saturated mercuric acetate in aqueous solution. The precipitate is separated by filtration. To 5ccs. of the filtrate are added 0.5cc. of a 1% alcoholic solution of naphthoresorcine and 5ccs. of concentrated hydrochloric acid. The mixture is warmed to boiling point for one minute and cooled rapidly in a stream of running water, an equal volume of ether is added and the whole is vigorously shaken. After separation the ether turns blue or violet if glycuronic acid is present. See Clogne and Fiessinger (2).

Roger (1) objects to the test on the grounds that it is negative in the presence of reducing substances, marked glycosuria and alcohol in the urine, whilst any impurity in the hydrochloric acid may further vitiate the value of the test; accordingly he has introduced the/

the following test:-

B. Roger's Test for Glycuronuria (3).

5ccs. of urine are placed in a centrifuge tube, followed by 0.2cc. of 1% ammonium hydroxide and 2ccs. of a commercial solution of subacetate of lead; a precipitate containing all the glycuronic acid falls out. The tube is then filled up with 1% ammonium hydroxide, centrifugalised, the supernatant fluid decanted, and the precipitate washed several times with ammonia. The purified precipitate is tipped into 5ccs. of distilled water, and the mixture poured into a test-tube. 0.5cc. of a 1% alcoholic solution of naphthoresorcine is added, then 5cc. of pure hydrochloric acid, and the whole placed upon a boiling water-bath for fifteen minutes, and subsequently cooled in running water. The resultant mixture is shaken up with 10ccs. of ether and the colour changes are the same as in the method of Tollen and Rorive.

The method is tedious, but it is to be used in all cases in which the urine contains a substance, which reduces Fehling's Solution. This test will recognize the presence of glycuronic acid in concentrations of 0.0002%, and as the urinary content normally averages 0.004% there is an ample margin of safety.

The physiological significance of glycuronic acid is set in a paper by Roger and Chiray (4) who claim that/



that the formation of this body is due to the anti-toxic function of the liver; the conjugation of the aldehyde group of glucose and glycogen was mentioned above and a list of the substances that can block the aldehyde and so allow of the oxidation of the alcoholic radicle and the formation of glycuronic acid may now be given.

Indol, benzol, toluol, phenol, skatol, thymol, menthol, camphor and its derivatives, acetanilide, nitrobenzol, naphthol; various alcohols; certain alkaloids such as morphine; antipyrine and pyrimidine; and the numerous endogenous physiological and pathological toxins.

Under normal conditions alimentary toxins - indol and skatol, reach the liver (which contains glycogen) and are conjugated with this body; oxidation of this substance postulates a previous fixation of toxin by the liver, and therefore the initial presence of toxins and a normal antitoxic function of the liver.

In actual practice the urinary glycuronic acid is usually conjugated with phenol, less commonly with skatol. A complex conjugation -ureido-glycuronic acid-urea sometimes occurs.

Roger and Chiray further applied their test to the study of glycuronuria in normal persons and to various types of patients. It was found that the acid in normal persons appears in largest quantity with/

with a meat diet, diminishes on a lacto-vegetarian diet, and disappears completely after prolonged starvation - the last phenomenon is explained by the depletion of the liver glycogen. They urge that usually the glycuronuria varies with the toxicity of the intestinal contents as modified by various dietetic regimes.

Clogne and Fiessinger (2) were induced by the work of the previous authors to study the occurrence of glycuronic acid at different times of the day, and their results may be tabulated as follows:-

(1) Morning Urine of Fasting Patient.

- (a) The urine of a patient who had previously urinated during the night seldom contained glycuronic acid.
- (b) The first urine of a patient who had not urinated during the night invariably contained glycuronic acid.

(2) Hourly Specimens after Food.

Glycuronic acid appeared at the second hour, reached its maximum at the third hour, and often disappeared at the fourth hour. These facts explain the findings in 1a.

The foregoing account of the normal glycuronic acid must be supplemented by a brief mention of the test of so-called "Provoked Glycuronuria" in which camphor is given by the mouth to provoke an outpouring of the acid. It was introduced by Roger and Chiray

(4), and applied to cases in which glycuronuria was absent; if the administration by the mouth of 0.5 to 1 gramme of camphor did not provoke its appearance they argued the liver insufficient.

To pass next to pathological considerations, and to the value of spontaneous and provoked glycuronuria as an index of hepatic derangement it will suffice to mention the conclusions of the various workers without going into detailed accounts of their case records.

At the outset it may be stated that there are three great classes of cases to be met with, leaving out of account altogether the degree of intensity of the reaction, viz:-

- |     |                                |                                |
|-----|--------------------------------|--------------------------------|
| (1) | Spontaneous reaction positive: | Provoked reaction<br>Positive. |
| (2) | Spontaneous reaction negative: | Provoked reaction<br>Positive. |
| (3) | Spontaneous reaction negative: | Provoked reaction<br>Negative. |

Roger and Chiray (4) found a diminution and ultimate disappearance of the acid in cases of Laennec's cirrhosis; in obstructive jaundice there was primarily an increased glycuronuria, and disappearance of the acid was seen to be a terminal phenomenon appearing about eight to ten days before death, and indicating hepatic failure. They explain the facts observed in this type of case by an ingenious argument that the cessation of biliary secretion produces a great increase/

increase in the intestinal putrefactive processes; the liver is thus deluged with endogenous toxins and an abundant production of glycuronic acid ensues until at last the liver cells become exhausted, conjugation ceases, and the disappearance of the acid heralds the fatal issue.

Chiray and Texier (5) came to the following conclusions:-

- (1) In diabetes mellitus the reaction is always negative and not provokable.
- (2) In Laennec's cirrhosis the reaction is absent at the time of the ascites but it reappears for several days after each paracentesis.
- (3) Obstructive jaundice has firstly a phase of hyperglycuronuria, followed by hypoglycuronuria and an *antemortem* aglycuronuria.
- (4) In Lobar pneumonia there are three phases:-
  - (a) In the febrile stage there is an intense reaction, thought to be due to increased liver activity, perhaps a part of the general antitoxic defensive mechanism.
  - (b) At the crisis the temperature and the glycuronuria fall together; this is interpreted as a cessation of the liver's antitoxic activity.
  - (c) During the post-critical period the reaction returns to normal.
- (5) Catarrhal jaundice.

(a)/

## (a) Icteric Period.

There is first a phase during which the reaction is normal, or in excess or normal, followed by a phase in which the reaction becomes weak and finally negative. This is taken to indicate the presence of hepatitis as the reaction was entirely different from that found in cases of obstructive jaundice.

## (b) Post-icteric Period.

Six or seven days are required for the reaction to become normal again.

Clogne and Fliessinger (2) came to the same conclusions but they also found a hyperglycuronuria in such diseases as biliary colic, amoebic dysentery and amoebic hepatitis, etc., and they use the expression liver reaction to explain this increased antitoxic activity.

Roger (7) discusses the test in relation to cases of intra-hepatic and extra-hepatic cancer but the paper is of little interest.

In a further paper the same author (8) quotes a case which illustrates the practical value of the test. A patient was admitted to hospital comatose, with sugar and acetone in the urine, and epileptiform convulsions. Roger found a positive glycuronic acid test, and quashed the diagnosis of diabetic coma that had been made, suggesting a nervous glycosuria and a starvation/



starvation kentonuria. Postmortem examination revealed a cerebral haemorrhage. The test is thus of great value in excluding pancreatic diabetes.

Barbier (8) applied the test in the prognosis of cases of atrophic infants, and found that absence of both a spontaneous and a provoked reaction always meant a fatal issue.

Roger and Chiray (9) and Gautier (10) confirm previous work, and the latter writer found that in provoked hyperglycaemia the glycuronuria did not increase. He concludes that a negative test and a negative camphor test mean irreparable liver damage and a fatal result.

Van Dooren and Destree (11) have applied the test more than 350 times using the method of Grimbert and Bernier; they have made certain modifications in the method of testing and they consider that the test is a dependable means of gaining an insight into the efficiency of the liver function.

Padilla (12) reviews the whole field of the tests of liver function and gives the accepted interpretation of the findings. He comments that none of the tests is reliable as the kidney function in the majority may modify the results in the blood and the urine. Bile itself he regards as a residual product with no important bearings upon the subject. The only liver test which throws light upon the prognosis is the camphor/

camphor test, and even it is not infallible, as he has obtained negative results with this test in cases of transient jaundice and other conditions in which there was no question of irreparable liver damage.

Chiray and Caille (13) in a purely chemical paper give an elaborate account of the technic of the camphor test of provoked glycuronuria into the details of which it is unnecessary to enter; they use a colorimetric scale made from a solution of pure glycuronic acid to estimate the quantity of the acid that is excreted. The dose of camphor they use is one gramme administered in a capsule.

Schmid (14) gives the history, reviews of literature, and gives his own experiences as to the value of the synthesis and elimination of combined glycuronic acid in affections of the liver. His opinion is unfavourable to the test. He was able to eliminate most of the inconveniences of the test by substituting menthol for camphor; yet the menthol test - as he found with the camphor test - was positive even in severe hepatic disorders. He is therefore inclined to regard the test as being of very little value in the elucidation of the functional capacity of the liver.

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CRITICISM OF THE TEST.

The tests of spontaneous and provoked glycuronuria are dependent for their presence upon several factors, (a) the presence of glycogen in the liver, (b) the presence of toxins - endogenous and exogenous, (c) a normal antitoxic function of the liver, (d) and perhaps a normal renal permeability, this point, however, has not received attention.

It has been shown that in diabetes mellitus glycuronuria is absent and its appearance cannot be provoked by the administration of camphor or menthol; doubtless this is due to the depletion of the liver's store of glycogen.

The absence of a spontaneous glycuronuria might be due to an absence of alimentary toxins, such as has been shown to occur on a vegetarian diet, in such cases the administration of menthol or camphor will decide the point.

In mild cases of hepatic disease the results of the tests are variable, there may or may not be a spontaneous reaction, but the provoked reaction can always be elicited: under certain circumstances there is an excessive excretion of the acid - the so-called "Hepatic Reaction".

If diabetes mellitus can be excluded absence of both the spontaneous and the provoked reactions seems to/

indicate a very grave liver insufficiency which usually has a fatal termination.

The test, then, is of definite value in arriving at a prognosis in cases of severe liver disease, but in the mild cases its sphere of usefulness is very limited.

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THE PHENOLTETRACHLORPHTHALEIN TEST.

As this test is probably the best one that has up to the present been discovered, as complete a review of the literature as possible will have to be given; the work has been done almost entirely in the United States of America.

The dye was first studied pharmacologically by Rowntree and Abel (1) in 1910, and amongst other properties it was found to be non-irritant when injected intra-venously but intensely so on subcutaneous injection. It was shown to be non-toxic, and it was almost entirely excreted by the liver into the bile-ducts and thence into the alimentary canal from which a little re-absorption took.

Whipple, Pughthal and Clark (2) and others used the substance as a test of liver efficiency, their argument being that it was only a sound liver that had the power of taking up the dye from the blood, and excreting it into the bile - the dye should all be recoverable from the stools. They endeavoured to estimate it quantitatively in 24 and 48 hour specimens of faeces, but the technique was both tedious and inaccurate, and a grave fallacy cropped up when it was shown that re-absorption took place from the intestine with subsequent re-excretion by way of the bile and continued for several days.

McNeill and Kahn, followed later by Aaron, Beck and/

and Schneider (3) and Williams (4) made a decided advance by endeavouring to recover the dye from the bile obtained by means of a duodenal tube. The last author applied the test to several cases of pregnancy toxæmias.

His method was to pass the duodenal tube on a fasting patient and after a flow of bile had been provoked by the Meltzer-Lyon technique 50 milligrammes of phenoltetrachlorophthalein were injected intravenously, and the bile collected over two minute periods in separate test tubes. The addition of  $\frac{1}{2}$ cc. of 40% sodium hydroxide solution produced a pink colour, and the time of the development of the maximum excretion of dye was taken as the criterion.

A series of twenty normal cases gave the average time as 16 to 24 minutes, and presumably a delay was taken as pathological, but as only two cases are quoted, one of convulsions with a maximum excretion time of 19 minutes, and the other a case of high blood pressure with a time of 28 minutes, it is unjustifiable to draw any conclusions as to this.

Piersol and Bockus (5) give a modified technique for the recovery and estimation of the dye after duodenal tubage, and advance the claims of their modified test.

These methods whilst showing a distinct advance over that of Whipple present the difficulties and uncertainties/

uncertainties of duodenal tubage, with no very accurate evidence of the reliability of the ultimate result.

Rosenthal (6) revolutionised the dye test by the publication of his first paper in 1922, in which he advocated the estimation of the amount of the dye in the blood. His first tests were performed upon dogs before and after prolonged chloroform anaesthesia, and he showed that whereas normal dogs exhibit the dye in their blood for a brief space of time, those in whom the liver had been damaged by the prolonged chloroformisation showed both a larger quantity in the blood, and this for a very much longer time. These facts were interpreted as being due to the failure of the liver to deal with the dye, and so it accumulated in the blood from which its sole outlet was by way of the liver.

In a second paper Rosenthal (7) was able to publish a further simplification of his previous technique, and the results of experiments on dogs which had been poisoned with phosphorus showed that there was marked elevation and prolonged of the blood dye curve. From several dogs whose common bile duct had been ligated normal curves were obtained, and Rosenthal urged the test as one of the liver efficiency and not of bile duct permeability.

In his next paper the same author (8) gives his final/

final technique as perfected for use in human beings, with the results of tests both in normal and in abnormal persons. It is of some importance to give a full account of this paper.

#### Technique.

The patient is weighed, and the amount of phenol-tetrachlorphthalein is calculated, 5 milligrammes per kilogramme of body weight being used. A syringe with a three-way tap is employed, one tap communicating with the needle, another with a vessel of normal saline solution and the third with the receptacle for collecting the specimen of blood.

A vein is punctured and 8ccs. of blood is withdrawn. The required dose of dye dissolved in 25ccs. of saline is drawn into the syringe, and injected. The vein-wall is finally washed with 25 to 30ccs. of saline as so irritant is the dye that failure to observe this precaution will often be followed by an acute phlebitis, accompanied by much pain and ending in a thrombosis.

The time is taken as soon as the dye is injected; with a small syringe 2 to 4 ccs. of blood are withdrawn from a vein in the opposite arm after 15 minutes, 60 minutes, and subsequently if necessary.

#### Determination of the percentage of dye in the blood.

The blood is allowed to clot spontaneously, and when/



when the clot has separated the serum is pipetted off into small test-tubes of uniform size, and one drop of 5% sodium hydroxide is added to each.

In order to prepare the standard solution for colorimetric estimations 10 milligrammes of the dye is dissolved in 100ccs. of distilled water, this is taken as the arbitrary standard as it represents the approximate concentration if all the dye remained in the plasma - that is 100% concentration.

With the sample of serum obtained before the injection of the dye a series of standards is made as follows:-

0.2cc.	0.2cc.	0.2cc.	0.2cc.	0.2cc.	0.2cc.
of	of	of	of	of	of
100%	100%	100%	100%	100%	100%
soln.	soln.	soln.	soln.	soln.	soln.
plus	plus	plus	plus	plus	plus
0.6cc.	0.6cc.	0.6cc.	0.6cc.	0.6cc.	0.6cc.
<u>serum</u>	<u>serum</u>	<u>serum</u>	<u>serum</u>	<u>serum</u>	<u>serum</u>
25%	20%	15%	10%	5%	3%

The unknown serum is now matched in daylight.

A qualitative test was also used by layering a specimen of serum acidulated with 3% hydrochloric acid over 5% sodium hydroxide. A ring is formed at the point of contact varying from blue to red according to/

to the quantity of dye contained in the serum. (Ring Test).

#### Normal Cases.

In 10 normal cases and 10 cases of extra-hepatic disease the following results were obtained and were taken as the criteria of normality:-

After 15 minutes there was a moderate trace to 6%, with complete visual disappearance in 30 to 45 minutes, but with a positive ring test after 60 minutes in 50% of the cases.

#### Abnormal Cases.

Five cases of carcinoma of the liver showed liver impairment with an average of 10 - 18% of dye for 3 to 5 hours, and a positive ring test in some for 24 hours.

Cirrhosis of the liver, chronic venous congestion of the liver, and certain toxæmias of pregnancy all showed abnormal dye retention and a study of these cases Rosenthal concluded that the test was simple, accurate and quantitative.

Rosenfield and Schneider (9) applied the test to pregnancy and its disorders, and not only were they able to confirm Rosenthal's standard of normality, but they were able to show varying degrees of liver impairment in hyperemesis gravidarum, and the hypertension group.

One of their cases is of interest both as confirming/

confirming the test and purely from the pathological standpoint; briefly it was a case of eclampsia which showed progressive liver impairment, succeeded by marked improvement but with a steady decline in the patient's condition and ultimate death from what the authors felt justified in diagnosing as uraemia.

They considered the dye test as of greater value than either the blood pressure readings or the clinical findings both in the diagnosis and the prognosis of these disorders.

Friedenwald and Gantt (10) produced a paper upon the recovery of phenoltetrachlorophthalein by means of duodenal tubage, and after a study of 93 cases they came to the conclusion that the test was of great value if meticulous care be taken in the details which they advised.

Gilbert and Coury (11) are apparently the first European workers to make use of Rosenthal's tests, this credit belongs to the medical service of the Hôtel Dieu of Paris.

Their conclusions are interesting, confirming those of the American workers as regards normal standards, and as regards the great value of the test in cases without jaundice of an obstructive nature; in cases of obstructive jaundice they obtained results which they could not interpret, as there was so marked a retention in the blood of the dye, very often lasting for 24 hours after the injection.

Deakin/

Deakin and Graham (12) after a review of the literature and an experimental research condemn the test of biliary tubage, chiefly on the grounds of the numerous factors which militate against the complete collection of the liver bile; attempts at a quantitative estimation are thus rendered futile, and the conclusions based upon them are meaningless.

Hoxie (13) compares the blood and duodenal tubage methods, and he finds that they do not give similar results; he is inclined to the view that the blood method of Rosenthal is by far the most accurate.

Bloom and Rosenau (14) have proposed a modification of the technique of estimating the amount of dye in the blood which promises to do away with the difficulties caused by haemolysis. It depends on precipitation of the dye by means of acetone, sufficient work has not yet been done to establish the accuracy of the modification.

Ottenberg, Rosenfeld, and Goldsmith (15) base their paper on the study of 100 cases; several modifications of Rosenthal's technique are suggested - 7mg. of the dye are used instead of the latter's 5mg.; and the omission of the first sample after 15 minutes, in this they have the support of Bogen (16).

They conclude that the test is useless in obstructive jaundice and unnecessary in mild catarrhal jaundice and other icteric conditions as there are simpler/



simpler ways of assessing the liver damage. On the other hand there were many liver conditions which the test served to elucidate, chiefly cirrhosis, metastatic cancer, and cardiac decompensation. Its greatest value, in the writer's view, is in the diagnosis of early liver metastasis, and in early cirrhosis.

In five cases of proved liver disease the test was negative, three were hepatic syphilis, one was a small cancerous metastasis, and a case of extensive secondary deposits of malignant disease which consistently gave a negative result.

A positive always meant a liver dycrasia, and in the absence of biliary obstruction a serious lesion. A negative test, however, did not always exclude such a condition.

After one hour the presence of 5% of dye in the blood was very suspicious, 8% was conclusive of a severe hepatic disorder.

Piersol and Bockus (17) carried out a series of comparative tests on a large number of patients and came to the following conclusions:-

(1) In 13 normal patients there was an average of 3 to 4% of the dye in the blood at the end of 15 minutes, and none at the end of an hour; it would appear that 1% at the end of an hour may be compatible with an intact liver.

(2) In 19 patients in whom the competence of the liver was/



was in doubt clinically, the authors' suspicions were confirmed - the majority of the group were syphilitic patients under treatment with arsphenamine, chronic cholecystitis and cholangitis cases or diabetics.

(3) In 23 patients in whom the clinical state indicated hepatic involvement - arsphenamine and tryparsamide jaundice, carcinoma, cirrhosis, etc. the Rosenthal test compatible with the surmised condition of the liver except in two cases. The amount of dye retention did not run parallel with the degree of jaundice, and there was considerable variation in this retention from time to time.

(4) In 12 cases a comparison was made between the excretion of the dye by the duodenal method, and they felt that the tubage method should not be abandoned, but should be the method of election when numerous veni-punctures were undesirable. This method measures the quantity of dye which the liver has been able to withdraw from the blood whilst Rosenthal's method estimates the amount left in the blood by the liver, so the two are really complementary.

(5) The principal disadvantage of the methods is an occasional thrombosis of a vein - the authors had five such accidents in sixty-seven injections - they suggest that the use of a smaller quantity of dye would obviate this.

It was noted that some of the patients with severely/

damaged livers developed a dull pain in the hepatic region either during or immediately after the injection, which lasted for a few minutes to a few hours.

Rosenthal (18) gives a brief survey of his pioneer work and indicates the lines along which progress has been made. Bogen (16) and he (19) have adopted a simpler means of estimating the dye percentages by means of a small comparator, using permanent standards for colorimetric comparisons; he also mentions Bloom and Rosenau's method of extraction of the dye by means of acetone, and estimating its percentage by direct comparison.

For experimental confirmation of the value of the test he quotes the work of Lamson (20) who used the test during his researches into carbon tetrachloride poisoning; for clinical confirmation the work of Ottenberg, Rosenfeld, and Goldsmith (15), Greenbaum and Brown (21), Bogen (16), Gilbert (11), Warfield and Youman (22), and Brown (23).

Rosenthal himself, in an experimental research upon fourteen rabbits found that deviation from the normal phenoltetrachlorophthalein curve was first observed after excision of 12% of the liver, and above this amount there was a close and constant correlation between the quantity of the tissue removed and the degree of functional impairment produced.

In a series of clinical cases his results were  
as/

as follows:-

(1) Syphilis - 24 cases - untreated.

Normal results were obtained in primary cases and tertiary cases with no manifestations apart from a positive Wassermann Reaction. 90% of the secondary cases, however, showed no liver impairment ranging from slight to moderate degrees.

(2) Arsphenamine Jaundice.

Eight cases were studied within two weeks of the appearance of jaundice, and all showed an extreme degree of liver impairment - 20 to 30% of the dye was retained at the end of one hour. The liver was found to become normal in  $2\frac{1}{2}$  to 3 months. In other cases it was found that whereas jaundice had disappeared in one to two months, liver function did not return to normal for two to four months, and the test is thus a better index of the state of the liver than is the presence of icterus.

(3) Catarrhal Jaundice.

In four cases during the height of the jaundice retention to the extent of 23 to 33% after one hour was found. The most marked cases of dye retention that Rosenthal has seen were in arsenical and catarrhal jaundice.

(4) Hepatic Carcinoma.

An average retention of 15% after one hour was noted.

(5) Cirrhosis - Portal, Syphilitic, and Pigmentary.

An average retention of 9% was found, and Rosenthal comments upon the mildness of the liver impairment in these cases.

In the discussion that followed the reading of this paper numerous workers confirmed Rosenthal's findings, and considered the test the best so far introduced. The general view was that the duodenal tube method was inaccurate as much of the bile escaped into the intestine and was not collected by the tube.

Walters of the Mayo Clinic said that the test was used on every case of obstructive jaundice, and in cases with marked retention operation was considered a bad risk. In these cases the bilirubin in the serum as estimated by the Van den Bergh method and the phenoltetrachlorophthalein retention varied directly and were closely correlated.

Levy and Morford (24) used the test in various conditions and came to the conclusion that departure from the normal curve always meant some degree of hepatic insufficiency.

Smith (25) used the test in twenty cases of normal pregnancy and in forty-four cases showing toxæmia - hypertension and albuminuria including eight women who probably had chronic nephritis and seven who had convulsions. The results obtained led to the conclusions/



conclusions that definitely abnormal retention of the dye in a patient with a toxæmia of pregnancy suggests that the toxæmia is a severe one and that it is one of the pre-eclamptic rather than the nephritic type, but the degree of retension of the dye does not appear to be an accurate index of the amount of actual necrosis of the liver. A normal test on the other hand is of doubtful value unless obtained a few days before delivery or if often repeated up to near the time of delivery. An accurate estimate of the practical value of the test must await further investigations.

The haemoclastic and phenoltetrachlorphthalein tests of liver function were used by Gonzalez and Karr (26) in groups of cases with primary or secondary liver disease. The comparative results of the tests are presented, with short comments on each group of cases presented. In many cases the pathological conditions of the organ were determined either at necropsy or at operation. It is said the haemoclastic reaction measures directly the physiological capacity of the liver cells, while the dye test measures this capacity dependent upon the patency of the biliary passages. The haemoclastic reaction appears to be more sensitive than the dye test, but a combination of the two is often helpful in the diagnosis or even prognosis of obscure hepatic disease. Emphasis is laid/



laid upon the large margin of safety of the liver, which precludes the use of any functional test to determine minor disturbances of the organ.

Kunfi (27) reports excellent results in fifty patients and controls with the test.

Bloom and Rosenau (28) undertook experiments to determine the fate of the dye after intravenous injection, immediately after the ligation of the common and cystic ducts and to correlate the findings with the Van den Bergh test. The experiments showed that in the absence of the liver and kidneys the dye remained in the circulation as long as the animals lived. After tying the common and cystic ducts, the liver was able to remove large quantities of the dye. The intravenous injection of the dye produced a transient hyperbilirubinaemia, as evidenced by the Van den Bergh test. The high incidence of thromboses and other untoward reactions is a serious objection to the use of this test in the wards. In no case of long-standing complete biliary obstruction have the authors been able to detect more than 4% dye retention in the blood stream.

Boardman and Schoonmaker (29) regard phenoltetra-phthalein as a valuable means of studying the functional capacity of the liver. Their preference is for the duodenal tube method whenever possible because of the objection on the part of the patient to the injection/

injection of large quantities of highly coloured material intravenously and because of induration and thrombosis with the large doses that are necessary in the Rosenthal method. With the duodenal tube method they recommend the adoption of the 50mg. dose as the standard, because there is no apparent advantage in the bigger doses. Because of the impossibility of collecting all the bile excreted over a given period quantitative estimations by the duodenal tube method are of no value. With 150mg. of the dye an initial appearance time of eleven minutes or over, would seem to indicate liver disease. In normal cases the time between the initial and the maximum appearance rarely exceeds three minutes.

Delprat and Whipple (30) regard the phenol test as unreliable as it is useless in cases of biliary obstruction and chronic inflammation of the biliary passages - "We cannot", they say, "rely in clinical diagnosis on any liver test which postulates a normal bile flow through normal bile passages".

Trainor (31) gives an incomplete account of the literature on the subject and reports the results of the test in twelve cases - his figures are similar to those obtained by other workers.

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CRITICISM OF THE TEST.

It would appear from a perusal of the literature on the subject that the phenoltetrachlorphthalein test is the most accurate test of liver function at present known to medicine; although it is actually the antitoxic function of the liver that is tested it seems justifiable to interpret the results in terms of total functional capacity - this point, however, requires further confirmation.

That the test is fairly delicate is shown by the clinical work that has been done - the finding of a definite dye retention in the blood in cases of secondary syphilis and in cases of this disease under treatment with the organic arsenical preparations, etc. - Rosenthal's experimental work (as yet, however, unconfirmed) shows that a definite dye retention in the blood begins after about 12% of the rabbit's liver is destroyed and progresses in proportion to the amount of liver destruction.

The trend ~~is~~ of opinion has been towards using the degree of dye retention in the blood as the criterion, rather than the dye excretion by way of the bile; the method of duodenal tubage precludes any possibility of an accurate determination of the amount of the dye which is excreted, and it can only indicate/

indicate the time at which the excretion begins, when it reaches its maximum, and when the excretion ends. No quantitative estimation of the functional capacity of the liver can, therefore, be achieved by this method, and as the results with Rosenthal's test will furnish an actual figure there would seem to be no doubt that this latter method is the one of choice in all cases in which there is no objection to repeated venipuncture. In such cases the duodenal tube method has a very definite place.

There are several objections, however, to the use of the test. The risk of venous thrombosis following the injections is not a negligible one, and the only workers (Piersol and Bockus) who have given figures as to its frequency had five such accidents in a series of sixty-five cases - that is  $7\frac{1}{2}\%$ ; the writer of this thesis made use of the test early in 1923, without, it is only fair to say, using the elaborate technique of Rosenthal, and in two of them a very painful but fortunately aseptic cellulitis of the arm resulted. From the point of view of the patient the risk is one which must be considered, and a very definite proportion of cases in which the test would be of interest will have to be excluded on account of the difficulty of guaranteeing a clean venipuncture - it is in women and children, of course, that this difficulty would be most frequently encountered.

The/



The second objection to the test is founded upon the statement of various workers (Gilbert and Coury, Ottenberg, Rosenfeld and Goldsmith, etc.) that the test is useless in obstructive jaundice and in inflammatory conditions of the biliary passages as the dye retention in the blood is so very high; the experimental work of Rosenthal and of Rosenau and Bloom would seem to prove the opposite. It is quite reasonable to reconcile the two views by supposing that, as in the case of renal or urethral obstruction and infection, in biliary obstruction and infection, a secondary liver insufficiency is produced, which would, of course, account for the dye retention apart altogether from the degree of biliary permeability. In support of the one view there is experimental evidence, whilst the other rests upon clinical evidence alone in the absence of pathological confirmation of the presupposed healthiness of the liver. Further experimental work is required to elucidate this point.

In conclusion it would seem that in the phenol-tetrachlorophthalein test we have a method by which a quantitative estimate of the functional capacity of the liver can be obtained with the risk to the patient of a venous thrombosis in, say,  $7\frac{1}{2}\%$  of cases, and a possibility that the condition of permeability and of inflammation of the bile passages may vitiate the results.

It is of note that there is no record in British literature of the use of the test in this country.



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THE HAEMOCLASIC CRISIS OF WIDAL.

As a test of liver function Widal introduced the now wellknown Test of digestive haemoclasia, or haemoclastic crisis; it may be as well to discuss at some length his papers upon the subject, before passing on to a short review of the subsequent literature.

There are two introductory papers of Abrami, Widal and Iancovescu (1&2) in which the principles of the method are expounded, and the experimental work upon which it is based.

The authors first showed experimentally upon dogs that after a meal of protein substances certain albuminous bodies are absorbed into the portal vein which are of much greater complexity than the amino-acids series, and are simply incompletely digested proteins; if the portal blood is withdrawn and injected into the peripheral circulation a protein or haemoclastic shock will occur, but at the same digestive phase systemic blood does not produce a like effect.

They deduce from these experiments that the liver has the function of arresting the noxious proteins and protecting the body from their effects - this function is the protein-fixing or proteopexic function of the liver.

Further they argue that a damaged liver is unable to detoxicate the portal blood after a protein meal, and that the incompletely digested protein escapes into the general circulation, and produces the post-digestive/

digestive haemoclastic crisis.

This phenomenon is a complex one, and is similar to that which occurs after the injection of Peptone; there may be noted a leucopenia, a hypercoagulability of the blood, a fall in blood pressure, and a lowering of the refractometric index of the blood serum.

To apply these facts to practice a simple method was evolved:-

The patient must have fasted for at least five hours, but preferably all night. A leucocyte count is made and then 200 grammes of milk are administered; twenty to twenty-five minutes later a leucocyte count is done.

Normally after a meal there should be a leucocytosis and if this is present the patient's liver is pronounced healthy; if, however, a leucopenia is produced it is regarded for clinical purposes as the indication of a haemoclastic crisis, and insufficiency of the liver can be diagnosed.

Widal in his communication (1) found the test negative in eleven normal people, and in thirty-four patients whose livers were not expected to be damaged; in an assorted series of thirty-one liver cases, catarrhal jaundice, Weil's disease, salvarsan jaundice, cirrhosis, etc. the test was positive in all but one.

In a third paper (3) the same authors note that if a second test is done within three hours of the first/



first a further leucopenia will not ensue - this emphasizes the importance of insisting upon a previous period of starvation.

A fourth paper (4) is of some interest as in it the results of the investigation of cases of so-called latent hepatism are given; it was found that for a period of about a fortnight after all the symptoms and signs had cleared up in a case of catarrhal jaundice a haemoclasia crisis could be produced, thus showing a prolonged and profound disturbance of liver function and confirming the modern view that the disease is a hepatitis rather than a choledochitis.

After injections of neosalvarsan a digestive haemoclasia was constantly found, and in various acute and chronic infections some degree of hepatic disturbance was shown to be present.

It is interesting to note that a positive result with Widal's test was usually accompanied by the presence in the urine of either bile salts or bile pigments, or of urobilin.

In a later paper by Widal, Abrami, and Hutinel (5) the effect of anaesthesia with various anaesthetics upon the liver was studied; chloroform was followed by a positive Widal reaction, ether was less constantly toxic, and nitrous oxide in two out of four cases was followed by a positive test with urobilinuria in one case and cholaluria in the other, novocaine had no effect/

effect upon the liver function.

Sömjen (6) in a study of eighty-two cases noted the rapidity of the appearance and disappearance of the leucocyte changes, in half of the cases the leucopenia ranged from 20% to 60% but it bore no relation to the severity of the liver derangement, and in two cases an enormous leucopenia was produced by simple massage of the liver. He is somewhat sceptical of the value of the test.

Powilewicz (7) in thirty cases of toxæmias of pregnancy showing hyperpiesis, eclamptic convulsions and retinitis found the test positive. He found that in pregnant women without signs of toxæmia 50% showed a positive reaction, and he is of opinion that some latent insufficiency of the liver was present.

Feinblatt (8) applied the test to eighty cases of normal adults taking leucocyte counts every twenty minutes for two hours; none failed to show a definite leucocytosis, and the curves were very uniform. A composite curve gave the following result:-

7379 before the milk and 8856, 9762, 9779, 9191, at half hourly intervals afterwards.

Widal's contention that alimentary leucopenia is to be regarded as pathological is thus confirmed.

Mazza and Traeta (9) recorded the blood pressure in thirty-four cases of parturient women after the ingestion of 200 grammes of milk. In 38% the pressure/

pressure fell, and in 38% of this group there is also a leucopenia. Thus of the total number 14.7% showed a haemoclasia crisis.

Brown (10) used the test in twenty-five miscellaneous cases of bilious headaches, migraine, constipation, gastro-intestinal upsets, etc. In ten there was a positive reaction, in five a suggestive one, but he was unable to differentiate the groups clinically.

Holzer and Schilling (11) in sixty cases in adults obtained a positive result in all obvious diseases of the liver, such as carcinoma, jaundice and cholelithiasis. In typhoid, paratyphoid B, scarlatina, measles and tuberculosis they also obtained a positive result in two cases. In cardiac diseases, patients under treatment with salvarsan, and cases of subacidity and anacidity a similar result was frequently found.

According to Mauriac (12) the test is inapplicable in children in whom be a normal post-digestive leucopenia.

In later paper the same author (13) disputes the value of the test as one of hepatic insufficiency. He declares that the number of leucocytes in the peripheral blood is so variable and fluctuates within so wide a range under physiological conditions that it is illogical to assume that post-digestive leucopenia is pathological until further observations have been made.

Monteleone (14) confirms the value of the test and/

and advocates it warmly.

Dahl (15) has applied the test in fifty men, women and children with and without liver disease; he also recorded the count in twenty-two other persons who had taken the test-meal. The findings in this latter group were so contradictory that he is inclined to deny the practical value of the test. The leucocytes increased in some of those with liver disease and dropped in others. The count seemed to vary irregularly from minute to minute even in fasting individuals who were kept absolutely quiet.

Andreen-Svedberg (16) found the <sup>test</sup> accurate in sixteen cases of certain liver disease and negative in doubtful cases.

Nussbaum (17) came to the conclusion that whilst a negative test was of no value in excluding liver disease, a positive result should lead to a very careful study of the case.

Adelsberger and Rosenberg (18) consider that a post digestive acceleration of blood sedimentation is of more value than a leucopenia; they found the test to give good results in liver insufficiency, whilst alimentary leucopenia depended to a large extent upon the vegetative nervous system.

Gerrard (19) during the course of a comparative study of the various methods of ascertaining the fact of liver damage in syphilitic patients undergoing treatment/



treatment with neosalvarsan, used the bilirubin content of the serum as his criterion. He found in ninety cases that no correspondence existed between the two tests and he feels justified in discountenancing Widal's Test, and regards it as quite unreliable.

Zehnter (20) gives a very full consideration of the test, and his paper merits more than a passing mention.

He first of all insists that the test is founded upon a hypothesis without scientific corroboration, namely that peptones are absorbed from the alimentary tract; further he challenges the experiments of Widal and his associates who produced a hemoclastic crisis by injecting portal blood into the peripheral circulation of animals during the post-digestive phase. Zehnter states that the injection of many bodies will have a similar result - viz. N.A.B., sodium bicarbonate, and sodium chloride.

out

He carried out numerous experiments upon the various factors that go to form the so-called haemoclastic crisis; he found that the blood viscosity was not constantly altered, that the refractometric index did not vary widely, that the fall in arterial pressure was frequently not observed, and that leucopenia was the sole constant factor.

As regards this leucopenia it can be produced by so many different causes - the application of heat, of cold, of electric currents, of painful stimuli, etc./



etc. that it cannot be taken as a reliable index of the occurrence of a haemoclastic crisis. Dorlencourt has shown that a post-digestive leucopenia is the rule in healthy sucklings.

The leucocyte formula is altered in haemoclastic shock in a totally different manner from that found in colloidoclastic shock, namely by the raising of the polymorph percentage, and it is doubted whether such a result could be brought about by the flooding of the body by peptones.

Turning to the practical difficulties of the test white cell enumeration is not so simple a matter as Widal would indicate, especially when a fall of 1000 cells per cubic millimetre is regarded as significant, and Zehnter is of the opinion that before any reliance can be placed upon the results it is necessary to count at least 10% of the cells, and it is quite inaccurate to have to use a larger multiplication in order to achieve the ultimate reading.

He quotes the following clinical results:-

<u>Aubertin</u>	50%	of	hepatic	cases	were	negative
<u>Adelsberger</u>	20%	"	"	"	"	"
<u>Erdsman</u>	50%	"	"	"	"	"
<u>Zehnter</u>	45%	"	"	"	"	"

Zehnter's analysis of his own cases is as follows:-

<u>DISEASE</u>	<u>TOTAL</u>	<u>REACTION.</u>	
		<u>Posit.</u>	<u>Negat.</u>
Hepatitis - various	10	9	1
Catarrhal Jaundice	12	6	6
Salvarsan Jaundice	4	0	4
Cancer of Liver	2	1	1
"Cardiac Liver"	12	6	6

His conclusion is that post-digestive leucopenia is not a sign of hepatic insufficiency, but probably depends upon the autonomic nervous system.

Legrand (21) gives an uncritical account of Widal's work, and his paper contains nothing of scientific interest.

Piersol and Bockus (22) compared Widal's Test and Rosenthal's Test in twenty-two cases, and found that the former was frequently at variance both with the latter and with the clinical condition of the patient.

In 44% of cases of slight or moderate impairment as shown by the phenoltetrachlorophthalein Test the haemoclastic crisis was negative; they, therefore, conclude that the test is quite unreliable.

Cipriani (23) made serial blood counts controlling the gastric secretion with a retention tube; he found a relation between hypersecretion and leucocytosis. Leucopenia seems to depend upon individual factors as well as upon a lowered acidity. He confirms Rachiusa's observation/

observation on the frequency of leucocytosis in hyperacidity and of leucopenia in achylia, even in fasting subjects.

Brack (24) examined the blood changes in sixteen cases of prurigo after the ingestion of milk or other substances, he believes that <sup>the changes</sup> he noted indicate a haemoclastic crisis in this condition.

Damato and Durante (25) produced almost regularly a haemoclasia crisis in tuberculous patients by the subcutaneous injection of minute quantities of tuberculin (down to a millionth of a gramme). The reaction was negative in healthy subjects.

Simon (26) noted that the irradiation of the pituitary body or of the midbrain modified the leucocyte count of the skin capillaries, producing especially an alimentary leucopenia. A notable diminution of leucocytes was manifest in 90% of all women with genital carcinoma. The phenomenon was negative in those who had recovered clinically. Hypervagotonia, the lower concentration of the calcium ions in the blood and reduced sensitiveness to epinephrin, cause dilation of the vessels with decrease of the leucocytes. The alimentary leucopenia may be of help in the diagnosis and prognosis of carcinoma.

Crainicianu and Popper (27) examined 47 cases of normal pregnancy during the month prior to delivery from the point of view of the functional efficiency of the/

the liver, the results of the haemoclastic crisis test will be dealt with here. They took <sup>the</sup> as standard of a positive test, a drop in arterial blood pressure of at least 10mm. of mercury and a fall in the leucocyte count of at least 2000 per cubic millimetre. Out of 20 cases the following were the findings:-

- (a) In 11 cases the blood pressure and the leucocytes remained the same.
- (b) In 2 cases there was a slight leucocytosis but the blood pressure fell 25mm. in one and 20mm. in the other.
- (c) In one case in which a typical crisis was obtained the patient had had a hydatid cyst removed from the liver in childhood, and they attributed the positive test to this fact.
- (d) In 7 cases there was a typical haemoclastic crisis.

The percentage of positive haemoclastic crisis tests in this series was thus 33% - a figure which they attributed to liver insufficiency occurring during the course of normal pregnancy.

Popper and Kreindler (28) assert that the changes in the sedimentation time of the erythrocytes during digestion are a valuable indication of liver function. They observed the varying speed before, and 45-60 minutes after the ingestion of 300-400 grammes of milk, fasting. Normal or lessened sedimentation time before the test, with an accelerated speed and augmented fibrinogen and albumin content of the serum after the milk, occurred in jaundice with a lesion of the liver parenchyma, in catarrhal/

catarrhal jaundice and in acute liver atrophy. Normal or increased speed before the milk test, associated with a lower speed, a diminished fibrinogen content, and an increased albumin content afterwards were noted in cirrhosis, in stasis from cardiac insufficiency, and in syphilitic jaundice and secondary carcinoma. They describe their technic, and emphasise their view that the method is more instructive in the diagnosis of insufficiency of the liver than the leucocyte count.

Barrow (29) has used the test in a large number of cases of enteric fever and he found that it was positive in 86%.

Mestre (30) found Widal's test positive in 40% of normal pregnant women (50 cases) during the last three months of pregnancy. Roch's methylene blue test was positive in 30%, and the two tests were concordant in 64%

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CRITICISM OF THE TEST.

It seems that Widal's Test cannot be admitted to throw light upon the functional capacity of the liver, and the early enthusiasm of the French writers has been replaced by scepticism, and finally by condemnation. Widal's contention as to the rationale of the test has received no confirmation, and all the evidence points to the fact that <sup>no</sup> protein substances of a more complex nature than the amino-acids are absorbed from the alimentary during digestion. Resting, therefore, on what must be regarded as a fallacy the test cannot be due to insufficiency of the proteopexic function of the liver, and on that ground alone is of no value.

It seems to be well established that - apart from infancy - a post-digestive haemoclasia normally occurs, but that under certain circumstances a leucopenia may result, but these conditions are not limited to cases of hepatic insufficiency, as 50% of liver cases show a normal leucocytosis, and a leucopenia may be caused by simple massage of the liver, by errors in gastric chemistry, and perhaps by disorders of the autonomic nervous system.

The technical difficulties of arriving at an accurate leucocyte count are considerable, as Zehnter has pointed out, and even a small error in any stage of the process may invalidate the result.

The/

The best criticism of the test will be found in Zehnter's paper and it is not possible, even in the light of more recent work to add anything to his conclusions - that the test is of no value as an index of hepatic efficiency.

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THE HAEMOCONIES TEST.



This section is abstracted from the monograph of Marcel Brulé (1) to which reference should be made for fuller details.

The Haemoconies Test purports to prove the passage or non-passage of bile salts into the duodenum, and it was founded upon the assumption that the bile and not the pancreatic juice is the essential factor in producing fat absorption. It is therefore necessary to dwell at some length upon the physiology of this part of the digestive process.

The classic works of Claude Bernard have established the fact that bile and pancreatic juice act simultaneously in the digestion and absorption of alimentary fats, but in the course of time the role of the bile has come to assume an inferior position, it being stated in many text-books that suspension of biliary secretion causes between 35% and 45% of the ingested fat to remain unabsorbed, whilst the suppression of the pancreatic secretion augments this figure to 75% or 80%, so that steatorrhoea is regarded as the sign par excellence of pancreatic insufficiency.

Brulé accepted this position unhesitatingly until with different collaborators from 1910 onwards he undertook the study of haemoconies, and was driven by the existing inconsistencies in the physiology of fat digestion and absorption to investigate the whole subject.

To/

To summarize his work briefly it may be stated that he showed that obstruction of the common bile duct prevents the absorption of fat into the blood stream with the concomitant appearance of haemoconies (2) whilst obliteration of the pancreatic duct or ducts has little influence in this direction (3), and he was later able to verify these results by using an accurate chemical method for the determination of blood fats (4,5,&6).

As regards the influence of the pancreatic juice on the absorption of fat, Brulé gives a fairly comprehensive review <sup>in</sup> his monograph (1), beginning with the early experiments of Claude Bernard upon rabbits which were taken as proving the paramount importance of this secretion. This work, performed in 1856, was followed by that of a series of other physiologists, who were, says Brulé, so obsessed with the idea of the infallibility of Bernard that they neglected any evidence which seemed to contradict his views.

Schiff (7) and Hedon (8), however, published experiments which were at variance with the current views, and physiologists began to form two schools of thought upon this subject. Rochaix (9) using a more delicate chemical technique found that ligation of the pancreatic ducts produced little difference in fat absorption, and this work was similar in its conclusion to that of Brulé and his co-workers.

With/

With regard to the influence of bile, Dastre (10) with experiments analagous in principle to those of Claude Bernard, came to a diametrically opposite conclusion, that is, that bile is the essential factor; subsequent workers showed that cessation of bile secretion into the intestine produced a profound diminution in the amount of fat absorbed.

It will be realised therefore that the subject of fat absorption was in a somewhat chaotic state at the time of Brulé's early work, and he was able to a large extent to explain the discrepancies, and to arrive at definite and apparently logical conclusions.

With his co-workers, Brulé showed up many of the fallacies of the previous experiments, for example, that it was futile merely to ligate the pancreatic duct, as it soon becomes permeable again unless a more radical operation such as a resection is done; that following a resection of these ducts a reflex cessation of biliary flow sometimes occurred (4); that it was incorrect to assess fat absorption by calculating the difference between the amount ingested and that recovered from the stools, because an accurate estimation of fat in the faeces is almost impossible, and some fat is always destroyed by bacterial activity in the intestines, whilst the gravest difficulty of all is the impossibility of being sure that the excreta examined are the result of the digestion or non-digestion/

digestion of the particular test-meal given.

As has been mentioned above their experimental work was made possible by the discovery of an accurate method of estimating the amount of fat in the blood (11,12), and it was shown that the non-passage of bile into the intestine almost prevented a post-prandial rise in blood fat, whilst occlusion of the pancreatic ducts was followed by a very slight diminution in the normal fat curve. They concluded that bile is indispensable to fat absorption and that if a rise in the blood fat does not take place after a fatty meal it can be postulated that there is failure of the bile salts to reach the intestine.

They were able to show that the presence of haemoconies was due to the absorption of fat, and that they were themselves fatty particles and whilst not representing the totality of the fat absorbed, they were an accurate index of its having taken place and to a certain extent of its degree.

To pass to the clinical side it was in 1910 that Lemierre and Brulé (2) first announced their "Epreuve des Hemoconies" as a simple procedure for judging indirectly the passage of bile salts into the duodenum. These bodies were first described by Raehlman, and it was later shown by Leva that a large number of them in the blood meant a raised fat content.

If a drop of blood taken one and a half hours after/



after a meal rich in fat be examined under the ultra-microscope a large number of highly refractile granules exhibiting Brownian movements may be seen - these are the haemoconies. They first appear about an hour after a meal, augment in number until two to five hours afterwards, and then they gradually disappear. After a meal very rich in fats they may persist for twelve hours or even longer.

It is possible to enumerate them roughly, according to the number in each field (13), and they are stainable sometimes with Sudan III. They have to be distinguished from the granules extruded from the leucocytes, but these are much larger in size, and tend to be clumped together in the neighbourhood of their crushed parent cell.

Haemoconies appear after the ingestion of any fatty substance but they do not seem to be provoked by other articles of diet.

Lemierre and Brule used as a test-meal a simple "petit déjeuner" of a roll and 50 grammes of butter, and they investigated a large series of icteric cases of the obstructive type. They found that if haemoconies appeared it meant a certain degree of biliary permeability; their absence was indicative of a complete obstruction. To some extent it was found possible to gauge the amount of bile salts that was reaching the duodenum.

They consider the test an excellent one for differentiating/



differentiating complete and incomplete biliary obstruction, and especially in distinguishing biliary calculus and carcinoma, as in the former the obstruction is variable whilst in the latter it remains complete and constant. The test is recommended by its simplicity of execution and the absence of discomfort to the patient.

In 1924 Gerrard (14) during the course of a research into liver disturbance during the arsenical treatment of syphilis carried out a number of comparative tests, using the Van den Bergh test as the criterion. He found that the haemoconies test was of no value in detecting mild degrees of impaired liver function.

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CRITICISM OF THE TEST.

The test of the haemoconies or blood dust, as the bodies are usually called in British and American literature, is one not of liver function but of biliary permeability; it is possible to conceive of so great a functional disorder of the liver that the excretion of bile salts into the intestine is arrested, but this must be a very rare occurrence and one in which no tests of liver function would be necessary to form a diagnosis, or more important still, a prognosis. As a test of permeability of the bile passages the test would appear to be a good one, but the work of Brulé and his collaborators needs confirmation before all of their contentions can be accepted.

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THE INDIGOCARMINE TEST.

Voelcker showed that in animals indigo carmine, if given in large quantities, is eliminated by both the kidneys and the liver, in smaller doses the kidneys alone take upon themselves the duties of excretion and none appears in the bile. Of the total amount that is ingested only about one quarter is excreted, the rest being destroyed by the organism. That which passes into the intestine by way of the bile is changed into a colourless chromogen, and re-absorption is minimal. By oxidation with mineral acids the chromogen, may be restored to its original colour.

With these facts in mind Hatiegaru (1) evolved a test of liver function.

#### Technique.

In the morning the fasting patient is intubated with a duodenal tube, and some of the duodenal contents are aspirated. 9.24 gm. of the dye is injected into the buttock dissolved in twenty cubic centimetres of normal saline, boiled, unfiltered, and warm. In normal cases 20 minutes after the injection the dye begins to appear in the bile and gives it a frank grass green colour, the excretion reaches its maximum in 2 to 3 hours, but continues to be excreted for about 10 hours. A colorimetric estimation of the quantity of dye is made by adding a known amount of the dye to some of the duodenal contents aspirated before the injection.

The/



The author found that the excretion of the dye <sup>disease</sup> was delayed in cases of liver without jaundice, but that in the presence of jaundice the test was valueless as no excretion appeared to occur. His conclusions are as follows:-

- (1) The dye is eliminated by the normal liver, and by the damaged liver in the absence of jaundice.
- (2) In diabetes the liver is badly damaged, and an excessive excretion of the dye takes place as the organ is simply acting as a filter.
- (3) In chronic venous congestion the rate of elimination is retarded.
- (4) In pernicious anaemia the rate is retarded and the total quantity is diminished.
- (5) In catarrhal jaundice, in Hanot's cirrhosis, in lithiasic jaundice, and in all diseases associated with jaundice the elimination by the liver is negligible.
- (6) Where duodenal soundage is impossible an examination of the stools might yield the necessary information.

Bossert and Loers (2) injected subcutaneously, with the duodenal tube in place, 5cc. of a 2% solution of methylene blue, or 5cc. of a 0.2% solution of indigo/

indigo carmine. Eighty two experiments were made in seventy - infants. In normal infants, with few exceptions, the methylene blue appeared in the bile in forty to sixty minutes, the indigocarmine in twenty to thirty minutes; with impaired hepatic function the elimination of methylene blue was accelerated, of indigocarmine retarded. The methylene blue test is more sensitive. In infants with grave tuberculosis or syphilis the methylene blue appeared most rapidly, and correspondingly the indigocarmine most slowly. Similar responses occurred in all cases of marked acute and some chronic nutritional disturbances. In four infants with pseudo-leukaemic anaemia, the reaction was approximately normal. In two cases of icterus neonatorum and one of infectious jaundice (colon bacillus) there was rapid methylene blue excretion, while in one case of icterus neonatorum the reaction to indigocarmine was normal.

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CRITICISM OF THE TEST.

Sufficient experimental and clinical work has not yet been done with this test to allow of any deductions as to its value being made. It has several grave objections - the necessity of duodenal tubage is undesirable, the attempts that Hatieganu has made to arrive at a quantitative estimation of the amount of the dye that is eliminated by the bile are, and the work of others has proved them, futile, a test which constantly gives positive results in the presence of jaundice irrespective of the cause must possess some grave fallacy, it cannot therefore be regarded as a test which will ever have more than a historical interest.

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TEST OF ADRENALINE HYPERGLYCAEMIA.



In a paper by Oeper and Verfy (1) this test of liver function is suggested; the liver has two glycogenic functions (A) an amyloplexic function by virtue of which it is able to fix the glucose which reaches it and store it as glycogen, and (B) an amyloptic function which enables it to convert the stored glycogen into glucose and set it free into the blood stream again. The writers believed that the amyloptic function had not been utilised in the researches upon liver function tests and they undertook a series of investigations into it by using adrenaline to provoke a hyperglycaemia, their idea being that if the liver was not functioning properly the response to this substance would be impaired.

Many authorities have disputed the exact mechanism of adrenaline hyperglycaemia and glycosuria, but all are agreed that it is due to a conversion of glycogen into glucose in the liver.

The patients were tested in a fasting condition in the morning, 1mg. of a carefully assayed suprarenal extract was injected into the buttock and specimens of blood withdrawn at intervals afterwards. The sugar content of the blood was estimated by a method which is not given. In a normal subject the rise at the different periods was found to be as follows:-

$\frac{1}{2}$ hour afterwards	+	0.73cg.	
1 hour	"	+ 0.43cg.	- this was the most constant figure.
2 hours	"	+ 0.01cg.	
3 hours	"	- 0.24cg.	

The plus signs mean a rise above the initial blood sugar, and the minus signs a fall below this figure. In all sixty patients were studied suffering from a variety of diseases.

The curves obtained from nervous, shell-shock, and hyperthyroid cases were very variable, depending largely upon their nervous condition. Cardiac cases showed no variation from the normal when their compensation was adequately maintained, whilst acute infections gave an increased response to the injection.

Twenty three liver cases were examined and the results may be set out as follows:-

<u>Catarrhal Jaundice.</u>	0.47cg. 0.71cg. 0.52cg. 0.33cg.
<u>Cholelithiasis and Cholecystitis.</u>	0.48cg. - average.
<u>Acute Liver Atrophy.</u>	0 - no rise.
<u>Cirrhosis - Atrophic.</u>	0.17 to 0.13cg.
<u>Cirrhosis - Hypertrophic.</u>	0.29cg. to 0.49cg.
<u>Haemolytic Jaundice.</u>	0.95cg. to 0.86cg.
<u>Malarial Hepatomegaly.</u>	0.82cg. 0.75cg. 1.08cg.

It will thus be seen that mild, benign jaundice shows/

shows a slightly increased provoked hyperglycaemia, haemolytic jaundice a very markedly increased one (due to an increased hepatic activity known as the hepatic reaction by the French authors), the reaction is below normal in cirrhosis, in severe anaemia, and in spirochaetal jaundice, and there is no rise at all in acute liver atrophy.

#### CRITICISM OF THE TEST.

As no confirmation of this test is available it is not possible to give a fair criticism of it; many factors would seem to be involved in an adrenaline hyperglycaemia and a number of control experiments would have to be done in patients with diabetes mellitus, hyperthyroidism, hypothyroidism, and various well authenticated disorders of the autonomic nervous system and of the endocrine glands before it would be possible to assess its value in liver diseases. The work that has been quoted above is very suggestive as regards the results in liver diseases, where the response to the injection seems to diminish with the severity of the functional disorder, but the matter must rest sub judice.

THE AZORUBIN S (A) TEST OF TADA AND NAKASHIMA.

The following is an abstract of a paper by Tada and Nakashima of the University of Kyoto (J.A.M.A. 25th October, 1924, p.1292) :-

After mentioning the various dye-stuffs that have been used in the study of liver efficiency and biliary permeability the authors decide that the ideal substance had not yet been employed. Tada (1) in a study of sixty-two dyes found that the ones which answered the requirements could be divided into three groups:-

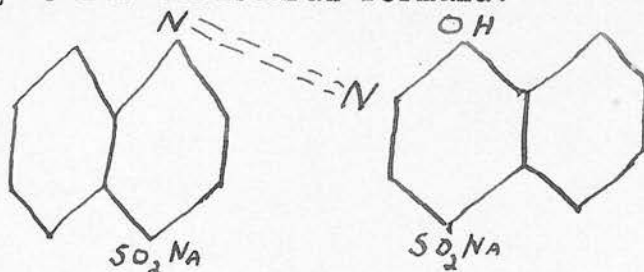
- (1) Those which only appears in abnormal bile - lithion-carmine.
- (2) Those which are eliminated only by the liver - First red A, benzpurin 10B, congo red 4R, benzo azurin G38-6.
- (3) Those which are eliminated in great part by the liver, with the rest in the urine - Eosin A, phloxin P, phloxin N.B.B., erotrosin B, scharlach R, ponceau 3R, and azorubin S (A).

They regard azorubin as the best, as the experiments of Tada (1) showed that a dye which is not normally excreted in the urine does not make its appearance there after the ligation of the common bile duct, whilst those dyes whose excretion occurs in/



in part through the kidneys become more abundant in the urine, and their excretion is markedly prolonged, after the ligation of the common duct or during some functional hepatic derangement.

Azorubin S (A) belongs to the mono-azo group, according to its structural formula:-



It is a dark red powder, easily soluble in water; when it is injected into the ear vein of a dog in 1% aqueous solution, 5% is excreted in the urine and 95% by way of the liver through the bile; it does not appear in the secretions of the stomach, pancreas, or intestines. That the dye is comparative innocuous was proved by an animal experiment: daily injections of 5ccs. of a 1% solution intra-peritoneally or subcutaneously for two weeks were unaccompanied by any clinical signs of intolerance and an autopsy was performed - there was no evidence of disease on microscopic examination of the liver and kidneys.

The following advantages are claimed for the dye:-

- (1) It is extremely harmless. \*\*\*
- (2) It is chemically stable and does not readily change colour.
- (3) Its appearance in the bile is easily determined owing to its colour and high concentration.
- (4)/

- (4) Renal disease does not influence to any appreciable extent its excretion in the bile.
- (5) In liver disorders and biliary obstruction its appearance in the urine is a rough index of the hepatic efficiency or the biliary permeability.

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TABLE OF COMPARATIVE TOXICITY.

Dye.	Minimum lethal dose per gram of Mouse.
Azorubin S. -----	3.0mg.
Phenoltetrachlorphthalein -----	1.0mg.
Phenolsulphonephthalein -----	1.5mg.
Methylene Blue -----	0.5mg.
Indigocarmine -----	0.4mg.

Results with normal subjects.

The dose that was found to be the optimum was 4ccs. of a 1% aqueous solution of the dye injected intravenously.

The duodenal tube was passed upon a fasting stomach, and when it was found to be in place, investigations were carried out along three different lines in a series of twenty-one persons with a total number of forty-two experiments. The results may be enumerated as follows:-

- (a) When no interference with the tube was made such as the injection of magnesium sulphate the bile that/

that trickled out was seen to become a deep red or scarlet colour in 30-50 minutes. This leaves out of account the dye which may accumulate in the gall bladder.

(b) 5 minutes after the injection of the dye 40ccs. of a 25% solution of magnesium sulphate was injected down the duodenal tube with the object of relaxing the sphincter of Oddi, and thus obtaining a flow of gall bladder bile (the B-bile of Lyon). In 17-18 minutes the bile became a deep red colour.

(c) Before the injection of the dye the gall bladder was emptied by means of the instillation of the usual dose of magnesium sulphate down the duodenal tube. At varying intervals afterwards in different persons injections of the dye were made - in one case injection of the dye was made  $1\frac{1}{2}$  hours after the gall bladder drainage and the coloration of the bile supervened in 25 minutes. In another case injection of the dye was made  $2\frac{1}{2}$  hours after the obtaining of the B-bile, and the bile became coloured in 70 minutes.

At the same time the urine was examined, and it was estimated that 5-8% of the azorubin was excreted through this channel, the dye remained in visible quantity in the urine for 6-10 hours.

The/

### The practical application of the method.

This aspect of the matter is the subject of a paper that is now in course of preparation by Nakashima. In the present paper the authors suggest the test as a reliable means of identifying the B-bile, and they base their suggestion upon experimental findings.

In twenty-one cases it was found that with the duodenal tube in situ the injection of 4ccs. of the dye solution produced a coloration of the bile for an average period of about 5 hours, they regard this as being due to the elimination of the dye from the blood serum by way of the hepatic cells directly through the bile ducts to the duodenum. If after the colour has faded from the bile the sphincter of Oddi is relaxed by means of magnesium sulphate a profuse flow of bile, stained a deep red resulted, this phenomenon they explain as due to the discharge of B-bile from the gall bladder. This is advanced as a method of obtaining with certainty specimens of the gall bladder bile, an absence of the second coloration being taken as an index of interference with the mechanism of emptying the gall bladder.

Matsuo (2) confirms these findings in a separate paper upon the origin of the B-bile.

### CRITICISM OF THE TEST.

As this test has not yet emerged from the laboratory stage criticism of it must be reserved until the publication of some clinical applications.

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THE ROSE BENGAL LIVER FUNCTION TEST.

Delprat (1) reports an experimental study of the dye rose bengal as a test of liver function. Rose bengal or tetraiodotetrabrom fluorescene is a non-toxic body of crystalloid form which remains in the circulation for an appreciable time after its injection. In the experimental work a technique similar to that adopted by Rosenthal in his researches upon phenol-tetrachlorophthalein was used, blood specimens were taken at 2, 4, 8, and 16 minute intervals after the intravenous injection of the dye, and the plasma content of the dye estimated by means of a colorimeter, ~~and~~ the standard being made up of blood plasma and a known solution of rose bengal. The experiments were done on dogs.

His conclusions are as follows:-

Rose bengal, when injected intravenously into normal dogs in a dosage of about 20 milligrammes per 20 pounds body weight remains in the blood stream for at least 16 minutes. There is a constant rate of elimination and the percentage concentration in the plasma at stated intervals may be traced out to form a curve - the normal curve of rose bengal retention.

The elimination of the dye from the circulation is profoundly affected by liver injury, when this injury is induced by chloroform poisoning, at any rate. With the experimental data at hand it is impossible/

impossible at this time to draw a correlation between the extent of the liver damage and the rate of elimination of the dye, and one would not be justified in claiming on the basis of the information thus far obtained that this method of experimentation will give any index of the functional capacity of the liver in any one of its numerous and complicated functions.

Delprat, Epstein and Kerr (2) use rose bengal in their study of liver function as a test medium. In normal persons, following the injection of a given amount of the dye into the circulation, the concentration of the dye computed under standard conditions of dosage and blood volume at four and eight minutes after the injection exceeds the limits of concentration of the normal cases. Complete biliary obstruction, which can be determined by other clinical tests, causes a retention of the dye in the blood stream. When it can be shown that the rate of elimination of rose bengal from the circulation, after injection, is reduced, or, in other words, when the concentration of the dye at four and eight minutes after injection exceeds the normal limits, a certain degree of liver dysfunction may be assumed to exist in the absence of complete biliary obstruction. A rough estimate can be formed from the concentration of the dye in the circulation at four and eight minutes after injection of the extent of the liver diseases.

CRITICISM OF THE TEST.

From these two papers the results of the test would appear to be very similar to those obtained with Rosenthal's test, no mention is made of the irritancy of the dye on intravenous injection. It is a point of great importance in deciding as to the necessity of introducing a further test of liver function so comparable to the latter. The matter must await the appearance of confirmatory evidence.

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TESTS FOUNDED UPON THE "SANGUINE" FUNCTION  
OF THE LIVER.



The fibrinogen test was suggested by Whipple and Hurwitz (1) and the following is an abstract of their paper in the journal of Experimental Medicine for January 5th, 1911, page 136.

During experiments on chloroform poisoning in dogs it was noted that many died after operations of uncontrollable haemorrhage; experiments to clear up this phenomenon seemed to throw light upon problems in connection with blood coagulation and liver function. They seem to indicate either that fibrinogen is formed by the liver or that its formation is dependent on that organ's functional activity.

When liver necrosis was produced by chloroform poisoning the amount of fibrinogen was found to decrease in direct proportion to the liver damage, till in complete necrosis it disappeared almost completely.

As the liver repairs itself the fibrinogen reappears *pari passu*, and when the liver is completely repaired an excess of fibrinogen is found to be formed, in accordance with Weigert's Law of tissue repair.

Whipple and Sperry (2) have shown that a liver does repair itself rapidly even after extensive chloroform necrosis.

The two other factors in blood clotting, thrombin and calcium, have been found not to vary in these conditions.

The/

The blood of the experimental animals does clot, but in the absence of fibrin the clot lacks body and stiffness, and is too weak to close the capillary vessels, etc., and thus arrest of bleeding is not effected. The amount of fibrinogen has to show a decrease of 10% before a tendency to haemorrhage appears.

The previous work upon this subject is quoted:-

Doyon and Kareff (3) and Nolf (4&5) have shown that extirpation of the liver causes profound changes in the blood, especially a disappearance of fibrinogen. In cases of phosphorus poisoning Corin and Ansiaux (6) and Loeb (7) show that both prothrombin and fibrinogen disappear together.

The present experiments differ in that thrombin remained normal even in most severe cases of chloroform poisoning.

Matthews (8) and Muller (9) found a parallel relationship between the number of leucocytes and the amount of fibrinogen in the blood, and they conclude that the source of the fibrinogen is the leucocytes and that the marrow is an important factor. The present experiments seem to show that marrow is not a factor, and that the leucocytes play no part. On the contrary there is a leucocytosis caused by the processes of liver necrosis and repair just at the time when/

when the fibrinogen is at its lowest percentage. Further the bone-marrow is not injured by the chloroform.

#### Method of estimating blood fibrinogen

It is necessary to include some account of the method used by Whipple and Hurwitz in order to be in a position to judge of its inapplicability to clinical work.

The dogs were starved for twenty-four hours before the experiments in order to ensure a clear plasma. The blood is withdrawn and oxalated, and then centrifugalised for half an hour. The plasma is pipetted off and carefully acidified with 5% acetic acid so that it just turns litmus red. 25ccs. of plasma is placed in a centrifuge tube and kept on a water-bath for 10 minutes at 60°C. The tube is submitted to prolonged centrifugalisation to throw down the flocculent precipitate, the supernatant fluid is poured through a weighed filter and the precipitate is broken down in the tube with 25 to 50ccs. of cold water, the mixture is again centrifugalised and the supernatant fluid poured through the filter; this washing of the precipitate is repeated several times until the supernatant fluid gives no precipitate with/

with silver nitrate.

The precipitate is then washed with 95% alcohol and carefully washed into the weighed filter, where it is finally washed with ether and allowed to dry in the air. It is then transferred to a desiccator over sulphuric acid for two hours, and dried in an oven at  $110^{\circ}\text{C}$  for two hours. The tubes are weighed subsequently at intervals of one to three days until a constant weight, correct within 0.2mg., is obtained.

This final weight is the amount of fibrinogen present in the blood examined, the normal amount in a healthy dog appears to vary between 0.300 and 0.600 grammes per 100ccs. of blood.

An experimental study upon a number of dogs in which central liver necrosis was produced by anaesthesia with chloroform for periods of two hours and over is then given; the authors conclude from this that the amount of fibrinogen in the blood is a very reliable index of the degree of liver damage.

In a further paper Whipple (10) draws a comparison between the haemorrhagic tendency of the dogs suffering from chloroform poisoning and the similar symptoms which are occasionally seen in cirrhotic patients, he suggests that blood fibrinogen determinations might prove of value in clinical work with this type of case.

Whilst admitting the impracticability of accurate estimations he advocates the use of simpler and rougher/

rougher tests, such as the observations of the consistency and amount of the clot in a test-tube.

Better still, a little oxalated plasma can be obtained and clotted, without blood-cells, by means of calcium salts thus giving a pure clot whose physical properties can readily be seen, and a rough estimation of fibrinogen is possible.

The average blood fibrin for normal men was found by Foster (11) to be 163mg. per hundred cubic centimetres; for normal women 179mg. per cent. The average plasma fibrin found for normal men was 332mg. per cent. The average blood fibrin for normal pregnant women was 273mg. per cent, and the average plasma fibrin was 414mg. per cent. Toxaemia of pregnancy (non-fatal) is associated with an elevated blood fibrin. The elevation seems to be parallel to the severity of the symptoms. The toxic manifestations due to nephritis complicating pregnancy are not accompanied by a marked fibrin elevation. It is inferred from the difference in the fibrin reaction that the toxic syndrome due to nephritis complicating pregnancy is of a different nature from that of a true toxaemia of pregnancy. Two fatal cases of acute liver atrophy were associated with a low blood and plasma fibrin content. Non-fatal arsphenamine jaundice was associated with an elevated blood and plasma fibrin content, lobar pneumonia was associated with a greatly elevated/



elevated blood fibrin, but the fibrin rapidly fell after the crisis. Complications (e.g. empyema) cause a subsequent fibrin rise, and the fibrin curve does not always run parallel to the leucocyte count. A series of fibrin determinations on a miscellaneous group of cases is tabulated.

Emile-Weil, Bocage and Isch-Wall (12) studied the various abnormalities in the clotting of the blood during the course of liver affections, their conclusions may most conveniently be dealt with under a series of sub-titles.

(a) The Non-retraction of the Clot.

In liver diseases the clot, as studied in two duplicate tubes to control each other, does not retract at all.

(b) Hypercholaemia.

The serum is much yellower than normal, even in the absence of jaundice.

(c) Crumbling of the Clot.

After twenty four hours the lower part of the clot begins to crumble, and numerous red blood corpuscles are released and form a delicate halo round the base of the clot (only, of course, observable when the clot has retracted sufficiently to allow a little serum to occupy this part of the tube).

(d)/

(d) The Aseptic Re-dissolving of the Clot. (Lefrou 13).

Normally a clot under aseptic conditions dissolves in about seven days. In liver disease this phenomenon is greatly accelerated, it may start in 24 hours and the process may be complete in four days. the authors used a special technique - 5ccs. of blood was placed in an Ehrlenmayer flask and allowed to clot with the flask lying on its side, after three hours the flask was placed upright on its base and a layer of paraffin placed over the blood in order to maintain asepsis and to prevent evaporation. Normally no solution took in 4-5 days. In liver cases at the end of 24 hours there was a heavy deposit of erythrocytes, and the clot began to detach itself from the side of the vessel, usually the solution proceeded until half of the clot was dissolved in 4 days and then the process stopped. Some cases of Laennec's cirrhosis showed a complete solution of the clot in 48 hours.

This phenomenon was present in all the hepatic cases which were examined for it.

(e) Diminution of the Platelets.

The normal platelet count was taken as 250,000 per cubic millimetre. In 18 liver cases (all advanced cases of various types) the platelet count was modified as follows - 10 cases were under 150,000, and only 2 cases approximated the normal count.

(f)/

(f) Lengthening of the Coagulation Time.

This was noted in certain cases of cirrhosis, of chronic venous congestion, etc., and the coagulation time increased to 16-45 minutes.

(g) Increased Bleeding Time.

Using Duke's method the authors thought that it would be possible to apply the facts they had observed by somewhat tedious laboratory methods to the clinique. They studied a series of liver by this technique.

- (1) Normal cases showed a bleeding time of  $3-3\frac{1}{2}$  minutes.
- (2) Catarrhal jaundice showed a variable time, but it was constantly and slightly increased.
- (3) In biliary lithiasis the time was little altered, but in the presence of chronic jaundice it was very slightly prolonged.
- (4) In cirrhosis it was not altered.
- (5) In cardiac congestion of the liver the time was prolonged to 16-24 minutes.
- (6) In acute alcoholic congestion of the liver the time was about 5 minutes.
- (7) In hydatid cyst of the liver the time was 4, 5, 6 and 7 minutes in one case at different times.
- (8) In neoplasms one was normal and the other showed a time of 5 minutes.
- (9) In Vaquez' disease there was a marked prolongation - 15 minutes.
- (10) In diabetes the time was normal.
- (11) In one acute case of malaria the time was prolonged to 6 minutes.

It/

It is essential to test the bleeding time on several occasions, and not to rely upon the information that is given by one examination. The authors regard the investigation of the sanguine function of the liver as an accurate method of ascertaining liver insufficiency in acute cases, in chronic cases the time is usually little altered and therefore little information may be derived from the investigations.

#### CRITICISM OF THE TESTS.

To deal in the first place with the work of the American writers upon the blood and plasma fibrin as an index of liver function; it has been fairly conclusively shown that injury to any tissue will cause a rise in the blood fibrin content, whilst in cases of severe and fatal liver injury experimentally produced in animals with chloroform or phosphorus the fibrin content falls. When these facts are applied to the study of cases in the clinique the experimental findings are confirmed, but in order to obtain a subnormal fibrin (blood or plasma) the liver must have sustained very considerable damage; in cases collected from/

from the literature acute liver atrophy seems to be the only condition in which this has occurred. The technique of the estimation is exceedingly laborious and the results do not warrant the adoption of the method in the solution of the problems encountered in the clinique.

Turning to the French work on the subject it will be of interest to quote the conclusions of Sutherland and Williamson (14) as to the blood changes in a certain type of purpura haemorrhagica (the essential thrombocytopenic type) in which some disorder of the spleen is probably the causal factor - they found (a) a great diminution in the number of blood platelets (b) a prolongation of the bleeding time from 10-20 minutes and (c) a non-retraction of the clot. It is not possible to say, therefore, that this blood picture is diagnostic of liver insufficiency as Emile-Weil contends.

To sum up, the sanguine function of the liver is one which is upset only in very grave cases, and the technique of the one valuable test is too elaborate to allow of its use in the clinique.

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BLOOD LIPASE.

The study of the lipase content of the various organs and tissues dates back to the end of the Nineteenth century, one of the earliest papers being that of Hanrot (1) who found that the blood had great lipolytic activities in order to enable it to make use of the fat reserves.

Loevenhart (2) in a paper upon the relation of lipase to fat metabolism studied the lipolytic activity of the tissues by making an extract of them and allowing it to act upon ethyl butyrate for a fixed time, the amount of butyric acid produced was an index of the quantity of lipase present. He found that the liver had a greater lipolytic activity than any other organ of the body, and that the blood serum had also very considerable amount. He regards lipase as a ferment with a reversible action, it can break down fat into fatty acids and glycerine, and in the body it can synthesise these products into fat. In states of malnutrition and starvation the blood and lymph become poor in fatty acid and glycerine so that the fat reserves are attacked by the lipase and can act as a fat store in the same way as it acts as a store for glucose and he would add to the functions of this organ the further one of "lipogenesis" and in certain pathological conditions this function may be disturbed.

Opie/

Opie (3) noted the presence of lipase in the urine of a case of acute pancreatitis, and Hewlett (4) made a similar observation in the experimental disease in animals. Von Hess (5) found the serum lipase to be uninfluenced by pancreatic extirpation, peritonitis, hyperthyroidism, thyroidectomy and ether anaesthesia. Bauer (6) described small fluctuations in the blood lipase in tuberculosis, cancer and syphilis.

Whipple (7) and Whipple, Mason and Peightal (8) contribute further to the knowledge of the subject, and the paper of the former is worthy of analysis. Whipple found small quantities of lipase in the urine of normal dogs, but a marked increase took place immediately after the injection of bile into the pancreatic duct for the purpose of producing an acute pancreatitis, this increase lasted for six hours or so. That it was not due to the pancreatitis was shown by the fact that it occurred after the simple opening of the abdomen, and the author attributed it to liver damage by the anaesthetic. From this point he was led to investigate poisoning with chloroform, phosphorus, and hydrazine, and in all of these conditions he found that the blood lipase was markedly raised - he gives tables to illustrate these findings. A series of experimentally produced diseases in animals was studied, and the results may be tabulated as follows:-

Eck's Fistula - this shows a rise immediately after the operation which rapidly settles down.

Acute haemorrhagic pancreatitis - normal blood lipase.

Acute intestinal obstruction - normal blood lipase.

The researches were next continued upon human beings the subjects of various diseases, with the following results.

Eclampsia. This disease showed a rise in the blood lipase, which was shown at autopsy to bear a direct relation to the amount of acute liver necrosis, mild cases showed little or no divergence from the normal.

Nephritic Toxaemia. Normal blood lipase.

Toxaemias and Hyperemesis Gravidarum. Normal blood lipase.

Various Liver Cases. Acute and fatal.

Cancer with focal necrosis, focal necrosis from various causes, leukaemias, chloroform poisoning, etc., all showed a high blood lipase.

Chronic Liver Disease and Chronic Icterus. These showed normal or subnormal blood lipase unless there were marked necrosis of the liver lobule. It would appear that chronicity of the disease affects that reaction against necrosis which in the normal or recently/



recently damaged liver so constantly brings about overproduction or escape of lipase. Obstructive jaundice often has a low blood lipase.

Various Diseases. Nephritis, some leukaemias, pernicious anaemia, diabetes, all showed a normal blood lipase; one diabetic case with lipaemia, even, had a normal blood lipase content.

Acute Diseases. Pneumonia, peritonitis, and various infections may raise the blood lipase to double the normal value or even more.

Cirrhosis. The readings in this disease depend entirely upon the presence or absence of acute liver necrosis, as in an exacerbation, late cases often show a low or subnormal reading.

### Conclusions.

The blood lipase in normal persons is maintained at a very constant level - 0.2cc. (vide infra), liver necrosis in a previously healthy liver causes a rise to 1.2cc. or more, that is five to eight times higher. In experimentally produced necrosis or after anaesthesia in the human being the rise continues for 12 to 24 hours, and by the 6th or 8th day it has fallen to normal as the liver has regenerated itself.

A great variety of acute intoxications not associated with acute liver destruction show no change in the blood lipase content. It would thus appear that a rise in the blood lipase is an index, not of liver disease, but of active destruction of the hepatic cells.

#### Technique of the Estimation of Blood Lipase.

Four tubes are prepared, each containing 1cc. of plasma or serum diluted with 4ccs. of distilled water and 0.3cc. of toluol to check bacterial activity. To two of the tubes is added 0.26cc. of ethyl butyrate, the others being used as controls. The tubes are shaken, corked, and placed in an incubator at 38°C for 18 to 24 hours. They are then cooled in iced water, 3 drops of azolitmin added and titrated in pairs to a neutral reaction using 1/10 normal acid and alkali. The control tubes usually show the blood alkalinity to be equal to 0.10cc. of 1/10 normal acid, and the butyrate tubes to be 0.10 to 0.20cc. above the neutral point with 1/10 normal alkali. This means that the total lipolytic activity is 0.02 to 0.30cc. 1/10 normal solution, that the lipase has split up the ethyl butyrate to that extent. We may then speak of the normal plasma lipase as 0.20 - 0.30cc. (always speaking in terms of 1/10th normal acid).

Sagal (9) gives a very full review of the literature up to 1916, but as the methods of the early observers/

observers were open to question it is unnecessary to quote the references that have not been summarised above. In an extensive series of 110 cases this author used the butyrate method and came to the following conclusions:-

- (1) The lipase content of normal serum is fairly constant.
- (2) Sera of patients suffering from bacterial diseases show a slight increase in enzyme activity, whilst sera from patients suffering from non-bacterial diseases show a slight depression of enzymatic activity.
- (3) Blood stained sera and sera from cases of hepatic cirrhosis show a marked decrease.
- (4) The lipase activity of the serum decreases with the advancing age of the subject.
- (5) The variations are so small as to be of no value clinically.

It may be noted that the author had very few liver cases and of these one only - an eclamptic - was acute, she showed a very definite increase.

Beumer and Fontaine (10) admit that the relation of blood lipase of fat metabolism is not yet settled. Whilst healthy infants usually have a high percentage of blood lipase variations sometimes occur. The most/

most marked decrease was found to be in relation to alimentary toxæmias, thus agreeing with the findings of Lust that in such conditions lipase disappears from the intestine. In anaphylactic shock the blood lipase is unaltered, and in tuberculosis the lipase values are not characteristic of the gravity of the disease. The cerebro-spinal fluid was found to contain no lipase even in the presence of lymphosis in pathological conditions.

Bach (11) found that the lipase content of the serum was not affected by the ingestion of food, nor could any rise be demonstrated after the patient had been kept for some days on a diet rich in fats. Intravenous injections of atoxyl - 0.15 - 0.2 gramme - reduced the lipolytic power of the serum by 60%, and the full activity was only restored after 22 hours.

Galdi (12) presents evidence that it is possible to reveal insufficiency of the liver in respect of fats by the precipitate that occurs when 2-3 drops of blood serum are dropped into a 5% neutral solution of glycerin. A whitish cloud forms in their wake, like a spiral of smoke, which gradually settles to the bottom as a flaky precipitate. This test differs essentially from the test of blood dust, both in the mechanism of its production and in its significance.

Simon (13) found lipases resistant to the action of quinine in the blood serum not only in affections of/



of the liver, but also in other patients. Rona's investigations on the different lipases in the blood are an experimental basis for further research.

Petow and Schreiber (14) confirmed the finding of a quinine resistant lipase in the serum of a large majority of patients with diseases of the liver and of the kidneys. Other patients were negative with the exception of two.

Vanysek and Felklova (15) used Rona's method for the differentiation of the organ lipases in the serum. They found the liver lipase in cirrhosis of the liver and in some cases of tuberculosis. Two patients with pernicious anaemia had only the blood lipase, young diabetics had the pancreatic lipase, whilst older ones gave the reaction attributed to the liver ferment.

Meyer and Jahr (16), after the examination of 187 patients state that they never found quinine resistant lipase in the serum of patients with normal livers and kidneys, nor with haemolytic jaundice. It seems to accompany progressive destruction of the liver cells, as in cholangitis and syphilis, but is not always present in hepatic disease in a not definitely progressive phase, as in cirrhosis and carcinoma. They assume that the finding of the quinine resistant lipase in the blood of patients with healthy kidneys indicates a recent destructive process in the parenchyma of the liver.



Mackenzie Wallis (17) has used the lipase content of the serum in the investigation of the effect upon the liver of the arsenical treatment of syphilis, and he reports that the results have been reliable.

Prewitt (18) entered into an experimental study of the isolated liver to elucidate the problem of the origin of the blood lipase, he was able to show that the liver is a source of ferment, and that certain substances added to the perfusion fluid have the power of stimulating the outpouring of lipase - amongst others, secretin has this property. His experiments are suggestive and they remove the reproach that the origin of lipase has not been studied.

#### CRITICISM OF THE TEST.

It would appear that an increase in the blood lipase is an index of active destruction of the liver cells, but not of a mere chronic liver insufficiency - the fallacy in the interpretation of the results is that any acute infection may double the blood lipase content. The finding of a quinine-resistant lipase in the serum appears to be very suggestive of disease of the liver or of the kidneys, and in the absence of kidney disease, to be almost pathognomic of liver insufficiency, it is not, however, always easy to exclude renal inadequacy in hepatic cases, and the value of the test is therefore much limited.

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THE URINARY AND BLOOD DIASTASE.

Since certain experiments purport to have shown that destruction of the pancreatic tissue leads to a great increase in the blood diastase it has been assumed by several workers - including Cammidge - that the liver is the source of this ferment, and that it is antagonised by the pancreas. Claims have been made for the interpretation of the blood diastase content as bearing some relationship to liver efficiency, a low percentage meaning a diseased liver. It would be well, therefore, to devote a section to this subject, as even a short survey of the recent work will serve to show how conflicting are the conclusions of the different observers.

Attention has long been focussed upon the diastatic activity of urine, more especially in pancreatic and renal disease, but it was not until accurate methods of measuring the blood diastase were introduced that any real progress was made; it may be well at the outset to mention the methods of examination, and then to tabulate the results of certain workers - this is not intended to be a complete review.

There are two principle methods, one in which a starch preparation is digested in different dilutions, iodine being used as the indicator of undigested starch; the other in which the amount of sugar formed by the diastase from a starch preparation is estimated.

(1) Wohlgemuth's Method and its Modifications.

Stock's (1) method may be given as an example, as it is applicable both to urine and to blood serum.

A 1 in 1000 solution of soluble starch is made up and kept in the ice-box to prevent decomposition. Twelve test-tubes are taken, and urine or serum measured into each as follows:-

Tubes:	1	2	3	4	5	6	7	8	9	10	11	12
Urine:	.5	.4	.3	.25	.2	.15	.1	.07	.05	.04	.03	.02

Into each tube 2ccs. of soluble starch are pipetted, mixed, the ~~tube~~ tube is transferred to a beaker at 40° C, and then to an incubator at 38°C. At the end of 30 minutes the tubes are removed and plunged into cold water, a few drops of N/50 iodine are added, and the colour changes are noted. Undigested starch is blue, completely digested starch is yellow, and the transition stages are violet to red.

The tubes showing no blue colour at all are those in which no starch remains, and the lowest one of these next to the purple one is taken as the limit; it contains just enough diastase to change 2ccs. of 1 in 1000 starch solution.

Calculation:-

x = number of ccs. of urine or serum required to digest 2ccs. of starch in 30 minutes at 38°C.



$D_{38^{\circ}.30}$  = no. of diastatic units per ccm.

$D_{38^{\circ}.30} = 2/x$

Supposing the last tube to be No.6.

$X = 0.15\text{cc.}$

Then  $D_{38^{\circ}.30} = 2/.15 = 13.3$  units.

Further details may be found in the papers of Corbett (2), Wohlgemuth and Noguchi (3), Harrison and Lawrence (4), the last writers used Dreyer's dropping pipettes for the measuring of the urine or serum and thus introduced a simplification.

## (2) Methods founded upon Sugar estimation - Blood Diastase.

Myers and Killian (5) estimated the amount of sugar formed from a known quantity of starch by means of Lewis and Benedict's method of blood sugar estimation. Fyfe (6) adopted the same principle, but he used Maclean's microchemical method, however, and this will be quoted at some length.

Into one of two 100ccs. Erlenmeyer flasks 1.8ccs. of 0.9 saline and 1cc. of 0.1% starch solution are pipetted, and into a control flask 2.8ccs. of 0.9% saline, 0.2cc. of blood is added to each, well mixed. The flasks are stoppered and placed on a water-bath for thirty minutes at  $37^{\circ}\text{C}$ . 21cc. of Maclean's acid sodium sulphate are added to each and the sugar estimated as in Maclean's method.

Calculation./

### Calculation.

The amount of starch used is one milligramme, and the difference between the sugar content of the two flasks measured in milligrammes will be equivalent to the amount of starch reduced to sugar.

E.G. Control: = 0.164mlg. Starch prep. = 0.259mlg.

The amount of the filtrate corresponds to  $\frac{4}{5}$  of 0.2cc. of blood, therefore the full amount of blood would contain 0.205mg. of sugar in the control, and 0.323mg. in the other. The difference, 0.118mg. of sugar is equivalent to the amount of starch transformed into sugar by incubating with 0.2cc. of blood, so that the diastatic index, or percentage of starch transformed, would be 11.8.

(Fyfe's calculation is somewhat difficult to follow, and it is doubtful whether the figures should really be multiplied by  $\frac{5}{4}$ ).

Cambridge, Forsyth and Howard (7) employed a different technic, and their blood sugar was estimated by Folin and Wu's method, their method has been severely criticized by Harrison and Lawrence (4).

### The Origin of Blood Diastase.

This is somewhat debated, Cambridge (7) brings evidence to show that the liver is its only source, whilst Davis and Ellison (8) conclude that the pancreas is its only source - between these two extremes the truth may ultimately emerge. The practical point is, however/

however, the claim that the liver manufactures the ferment and that it is destroyed by the activity of a normal pancreas, so that the advocates of this theory use the level of the blood diastase as a test of liver function. Whether their claim rests upon a solid foundation is yet unproved, but it may be of interest to examine the results of the various workers.

Harrison and Lawrence (4) were able to show that the plasma and serum had the same diastatic content, that the blood diastase remained constant throughout the twenty-four hours, and that the diastase and sugar curves did not correspond, as Cammidge has stated; these observers stress the importance of estimating both blood diastase, urinary diastase, and the number of units excreted in the twenty-four hours if accurate conclusions are to be drawn from the work.

It will be found of interest to tabulate the diastase values in normal and abnormal conditions as published by the different observers.

(1) Harrison and Lawrence (4,9) - Wohlgemuth's method.

(a) Renal Disease.

There is a defect in diastase excretion, with a raising of the blood diastase only in those cases in which more than three-quarters of the kidney substance ceases to function. Blood = 10 units. Urine = 6.7 units is the average finding in a case with both diminished excretion and retention.

(b)/

(b) Normal Cases.

Blood = 3-10 units:    Urine = 6.7-33 units:    Total  
in 24 hours = 8000 to 30,000 units.

(c) Pancreatic Diseases.

During active destruction of the pancreas (acute pancreatitis) or obliteration of its ducts there is a marked increase in both urinary and blood diastase; a combination of a high urinary index and a high blood index is indicative of acute pancreatic disease, and may indeed be regarded as pathognomic. A high blood index alone is highly suggestive.

(d) Diabetes Mellitus.

In 48 cases 14 showed a subnormal blood diastase (1-2 units). One showed an increase (13.3 units), and the remainder were normal within normal limits. The urinary diastase was subnormal owing to the co-existing polyuria, but the daily excretion was not diminished.

(e) Liver Disease.

TABLE.



<u>Case</u>	<u>Blood Diastase</u>	<u>Urinary Diastase</u>	<u>Total Diastase</u>	<u>Remarks.</u>
1	1	23.5	16,9000	Malignant disease of the liver.
2	1	10	?	Carcinoma of the liver.
3	2	23.5	8,500	Catarrhal Jaundice.
	8	10	12,000	Same case - convalescen
4	3.3	8	4,3000	Cirrhosis of the liver.
	8	10	7,800	Same case - clinically improved.
5	7	16.7	12,000	Cirrhosis of the liver.
6	5	16.7	17,000	Melano-sarcoma of the liver (secondary).

Twenty cases in all were studied, but the majority showed a normal diastase content. In three severe cases the blood diastase was very low and in two who improved clinically it rose subsequently.

These authors conclude that the chief value of the estimation of diastase is in cases of acute pancreatic disease where the findings are characteristic.

(2) Fyfe (6). Sugar Formation Method.

(a) Normal Cases.

Blood diastase = 8.6 units.

(b)/



(b) Diabetic Cases.

Blood diastase = 24, 14.5, and 3.5 units in three cases.

(3) Myers and Killian (5). Sugar Formation Method.(a) Normal Cases.

Blood diastase = 15-17 units.

(b) Diabetic Cases.

24-75 units varying with the blood sugar.

(c) Pancreatic Disease.

Blood diastase = 45 units.

(d) Nephritis.

Blood diastase = 13-44 units: average = 30 units.

(e) Syphilitic Cirrhosis of the Liver -

One Case. Blood diastase = 15 units.

The authors conclude that the blood diastase is raised in nephritis, pancreatic disease and diabetes.

(4) Stocks (1) Wohlgemuth's Method.(a) Normal Cases.

Blood diastase = 6.6-8 units: urinary diastase = 10-13.3 units.

(b) Diabetic Cases.

In 4 cases the blood diastase was 8 units, and in one case it was 28 units.

## (c)/

(c) Pancreatic Disease.

His findings agree with those of other writers.

(d) Liver Disease.

- (1) Cirrhosis: 10, 13.3 10 units. (three cases).
- (2) Chronic Venous Congestion: 13.3 and 13.3 units. (two cases).

Dodds (10) in a chemical research upon the urinary diastase came to the following conclusions:-

- (1) The original technique for the diastase reaction is criticised because it takes no account of the varying reaction of the urine.
- (2) It is proved that the optimum reaction for urinary diastase in the presence of phosphate was pH6.1, as in the case of the salivary diastase.
- (3) A modified technique is described whereby the urine is diluted with a phosphate buffer solution, thus bringing all urines to the optimum pH before the starch digesting power is tested.
- (4) Ammoniacal decomposition, by making the urine more alkaline, decreases the diastatic power as determined by the old method, but has no effect by the method suggested.
- (6) Diastase tends to cling to urinary deposits hence all urines should be shaken well before the estimation is made.

Mackenzie Wallis (11) studied the urinary diastase content by means of a modification of Wohlgemuth's method in normal pregnancy and the toxæmias of pregnancy. In normal pregnancy the diastase was between 10 and 33.3 units; in nephritic toxæmia the content was subnormal, and in ordinary, presumably hepatic, toxæmias it was very high, and only in acute pancreatic disease has the author found higher readings. He does not record his figures.

#### CRITICISM OF THE TEST.

Dodd's work upon the pH of the urine and the diastatic activity has vitiated the results of the previous observers, and the figures that have been quoted must be interpreted with reserve. The influence of the kidneys upon the excretion of diastase limits the applicability of the test in liver cases to those in which the renal function is normal - a difficult question to prove in these cases.

As to the urinary diastase in various pathological conditions, the only proved effects are in acute pancreatic disease where the content is very high, and in renal diseases where there is ~~is~~ a diminution of the excretion. There is no consistency in the findings in liver disease.

Turning to the blood diastase, the estimation of which is subject to few objections, the thesis that a low/

low reading is due to liver insufficiency has not been sustained.

The study of diastase remains an unprofitable field as far as the elucidation of liver efficiency is concerned.

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THE METHYLENE BLUE TEST.



In 1912 Roch (1) of Geneva introduced the Methylene Blue Test of liver function in a paper read before the Societe Medicale des Hospitaux de Paris.

At his instigation Syrtlanoff (2) had carried out some experiments with this dye, and has shown it to be eliminated by both the liver and the kidneys, the route of elimination depending upon the dose of the dye and upon the method of administration. After ingestion by the mouth 0.05 gm. is excreted more quickly and in larger amount than a much greater quantity injected, and more especially in patients who are the subjects of liver disease. By progressively diminishing the dose they found that there was a threshold below which excretion in the urine did not take place; it seemed that a dose of 0.002gm. was about the maximum amount that a healthy adult could absorb without any colour changes occurring in the urine.

Roch suggests that the liver is able to fix a certain quantity of dye if it is functioning normally, and he quotes experimental work by other observers confirming this general property of the liver towards dyes such as methylene blue and methyl violet; it is probably part of the antitoxic function of the organ. Any diminution in the capacity of the liver to fix such a dye might be interpreted as pointing to a functional derangement, and accordingly Roch proposes the fixation of methylene blue as a test of liver function, /

function, at the same time simple and accurate and devoid of discomfort to the patient, unlike many of the tests in use at that time.

The presence of a green coloration in the urine after a dose of the dye which should normally not appear in the urine, is taken as the standard.

#### Technique.

The patients were on the usual hospital diet (7 a.m. cafe au lait; 10 a.m. soup or milk; 12 noon meat, vegetables, stewed fruit; 4 p.m. cafe au lait or milk; 6 p.m. soup). At 8 a.m. they received two powders each containing lmg. of methylene blue in a draught of water, and four urinary specimens were collected at stated intervals afterwards, viz. 1st 8 a.m. to noon; 2nd noon to 4 p.m.; 3rd 4 p.m. to 8 p.m.; 4th 8 p.m. to 8 a.m. It was found later that the night specimen was unnecessary and that the specimens between the hours of noon and 4 p.m. was the essential one for all clinical purposes. The amount of methylene blue was estimated colorimetrically in this specimen, the results being expressed as milligrammes per litre.

By experiment it was found that an amount of 2 decimilligrammes in the urine gave rise to no colour changes, but double this amount causes a definite green colour which was noticeable at a glance.

Occasionally/

Occasionally the original colour of the urine made it impossible to detect the presence of the dye - in these cases it was sometimes possible to clear the urine with lead acetate, but bilious urines could not be treated in this way so that the results in such cases were not to be depended upon, if indeed they were of use in the presence of obvious signs of hepatic derangement.

In a series of twenty liver cases Roch demonstrates the applicability of the test, and he considers that with certain reservations, a green colour in the urine after a dose of the dye such as he gave is an indication of functional damage to the liver.

There are three factors which militate against the accuracy of the test (1) disorders of absorption from the alimentary canal (2) renal disease in which a definite retention of the dye takes place, (3) the excretion of the dye as a leuco-derivative.

- (1) Roch did not encounter a case in which delayed absorption due to some intestinal cause could be proved, but he admits the possibility.
- (2) In several cases of cirrhosis which at one time gave a positive test attacks of renal impairment with albuminuria and oliguria were found to be associated with an absence of excretion of the dye. This Roch regards as a serious objection, as so many of the liver cases have sustained kidney damage.

(3)/

- (3) In only one case did the dye become converted into a leuco-derivative, although this possibility was kept in mind in all cases and precautions adopted to detect the dye in its changed form.

Hatieganu used methylene blue as a test of liver function in a somewhat different way. He collected the bile by means of a duodenal tube, and endeavoured to detect colour changes in it in various morbid conditions. His work is not worth quoting at any length as he came to the conclusion that the dye was of absolutely no value if used in this way as the coloration that it imparted to the bile was indistinguishable from that of biliverdin in all liver conditions as well as in the normal subject. In diabetics, however, the bile is coloured blue as if there were a hyperpermeability of the liver parenchyma to the dye.

#### THE TEST OF INTERMITTENT GLAUCURIA.

This is probably the first colour test of liver function and it was introduced in 1898 by Chaufford, Castaigne and Cavasse (4). After the subcutaneous injection of methylene blue the dye begins to be eliminated at the end of half an hour, reaches a maximum in from three to four hours and then begins to/



to disappear, but the final disappearance does not occur until about the sixtieth hour. In liver cases the elimination is intermittent, but as the role of the kidney cannot be excluded the test has completely fallen into disfavour.

Cohn (5) tested 15 healthy persons and 45 patients without either hepatic or renal disturbances with Roch's methylene blue test; fully 60% of the healthy persons and 80% of the patients responded with a positive reaction. They therefore consider the test as useless.

Rosenthal and Galkenhausen (6) used the excretion of methylene blue in the bile as a test of liver function. In all forms of icterus examined the flow of bile was not markedly impaired, and in all affections of the liver in which parenchymatous injuries characterised the clinical picture the excretion of methylene blue was considerably accelerated. Whereas under normal conditions the excretion of the dye through the bile begins in from 55-75 minutes after its injection, in a few cases it is delayed until 90-95 minutes; in severe affections of the liver cells the bile became coloured with the dye in 15-35 minutes after the subcutaneous injection of the methylene blue. It is therefore evident that the diseased liver cells are characterised by an unusually high permeability for methylene blue, as compared with the normal cells.



Hamid (7) tested in 30 persons the excretion of methylene blue in the bile. He found that there was no difference in the time of its appearance between the healthy subjects and those with liver disease.

The present writer's own series of cases in which Roch's test was used confirmed the findings of Cohn. (See the special chapter).

#### CRITICISM OF THE TEST.

There are two methylene blue tests, one in which glaucuria is sought, and the other in which the excretion of the dye in the bile is ascertained by duodenal tubage.

The first method was used by Roch and by Chaufford; Roch supposed that after the ingestion by the mouth of a small quantity of the dye the liver is able to fix and excrete all of it in the bile - in conditions of liver insufficiency this fixation and excretion does not occur, and the burden of excretion falls on the kidneys, the urine showing the presence of the substance by the assumption of a green colour. Little confirmation of the claims of these writers has been forthcoming, and the results of other workers have quite discounted the value of the test, as it is positive in a large proportion of healthy people, and any impairment of renal function prevents the excretion of the dye in the urine.

That/

That the test is valueless is shown by the results of the examination of the excretion of the dye, after subcutaneous injection, in the bile collected by duodenal tubage; conditions in which the liver function is known to be impaired are associated with a greater and more rapid excretion of the dye, a finding which has received ample corroboration. Roch's test is based upon a fallacy, as no retention of the dye in the blood is conceivable under these circumstances.

The duodenal tube method requires further experimental work upon it before it can be accepted, as so anomalous a fact as a hyperpermeability of the diseased liver cells to a dye is not in consonance with the behaviour of any of the other dye stuffs that have up to the present, been used - it is not clear upon the insufficiency of which liver function the anomaly depends.

To sum up, Roch's and Chauffard's tests are without value in the elucidation of the functional capacity of the liver, and the duodenal tube method gives results which are not comparable with those of the other dye tests.

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THE ABDERHALDEN REACTION IN RELATION TO  
LIVER DISEASE.

ABDERHALDEN described in the blood of pregnant women a defensive ferment against the placental albumins, and other German writers have described similar defensive substances that protect the body against the protein of the thyroid in Grave's disease, and against the protein of cancer tissue in cancer patients. Noel Fiessinger (1) in 1908 described similar substances in the serum of persons with acute liver degenerations such as occur in the terminal stages of a cirrhotic patient. The serum of these persons acts towards liver protein in the same way but the specificity was not absolute.

He aptly says "The patient no longer defends his liver, but defends himself against his liver".

Fiessinger and Broussole (2) describe a case of toxic jaundice in which they performed the reaction of Abderhalden at various times during its downward progress, at first they found that the serum contained protective substances against liver protein, but later further substances developed which were protective towards the proteins of the kidney, the thyroid, and the suprarenal glands. They claim that this is the first time that the reaction of Abderhalden has been applied to a case of toxic jaundice of this severity - acute liver atrophy.

Robin, Fiessinger and Broussole (3) continued their researches upon this subject in a series of cases/



cases illustrating various types of liver disorder, they give a full description of their technique, and they divide their cases into certain categories:-

(a) Acute Jaundice - 8 cases.

This was a group of varying etiology, catarrhal, alcoholic, toxic, gallstones, etc. Two of the cases gave a positive reaction, one of them a post-typhoidal jaundice and the other a lithiasic case.

(b) Chronic Jaundice - 4 cases.

Two of these gave a positive reaction on several occasions.

(c) Acute Liver Atrophy.

Their previous case is quoted.

(d) Cirrhosis - 7 cases.

3 cases of Laennec's cirrhosis. One pos., One neg., one doubtful.

3 cases of Hanot's cirrhosis, all doubtful.

1 bronze diabetes. The liver was positive on one occasion and the suprarenals were positive on three occasions.

(e) Cancer. 2 cases.

Both gave negative results.

(f) Chronic Venous Congestion of the Liver - 3 cases.

All gave negative results.

(g)/

(g) Controls.

These all gave negative results, except one case which on further investigation was found to have a damaged liver.

The authors conclude that a positive test means that there is active destruction of the liver parenchyma going on, but a negative test is of little value as the results vary from day to day in an inexplicable way, perhaps as the lesion which is responsible for the liver destruction progresses or retrogresses. The reaction is of interest, but the authors admit that it is quite inapplicable to the clinique. They quote confirmatory work by Hertz and Brokman (4).

CRITICISM OF THE TEST.

The presence of an Abderhalden reaction is found only in cases of liver disease in which there is an active destruction of the parenchyma - the finding of a positive reaction is not in these cases at all essential to the diagnosis, and the difficulties of the technique and the variability of the results preclude the use of the test in the clinique.

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RECORD OF PERSONAL RESEARCHES.

LEVULOSE TOLERANCE TEST.

BLOOD DIASTATE.

SALICYLATE OF SODIUM TEST.

METHYLENE BLUE TEST.

THE LEVULOSE TOLERANCE TEST IN PREGNANCY.



The following series of experiments was carried out at the Royal Maternity Hospital, Edinburgh, during the summer of 1923, and chemical analyses were performed at the Laboratory of the Royal College of Physicians, Edinburgh, by the writer. The kindness of the late Dr Lamond Lackie and Dr William Fordyce in placing their cases at his disposal on the one hand, and of Lt.-Col. McKendrick and Mr Kermac for permission to use the chemical laboratory of the College on the other hand must be gratefully acknowledged.

#### Technique.

As the method of Spence and Brett without modification was adopted it is unnecessary to do more than give a brief recapitulation. The patients were allowed no nutriment of any description after their supper of the previous night, relaxation of this rule was only permitted to the extent of a glass of water, if the patient desired it.

At 8 a.m. a specimen of blood was taken either from the finger into one of Maclean's capillary pipettes, or from a vein by means of a hypodermic syringe and a very fine needle. Immediately after the withdrawal of this specimen a solution of levulose was administered by the mouth, the dose being/

being regulated according to the weight of the patient, as follows:-

A patient weighing	80kilos.	received	50 grammes	of levulose
"	"	60kilos.	"	40 " "
"	"	40 kilos.	"	30 " "

At subsequent intervals of half an hour further specimens of blood were taken, that is at 8.30 a.m. at 9 a.m. at 9.30 a.m. and at 10 a.m. In all therefore five specimens of blood <sup>were</sup> taken; it may be added that each specimen was duplicated in order to minimize the possibility of an error either in the obtaining of the blood or in the actual chemical manipulations which had to be performed.

As a bio-chemical laboratory was not attached to the hospital, it was impossible to carry out the chemical analyses in the intervals between the taking of the samples, and this part of the test had to be delayed until the tubes could all be removed to a laboratory. Much time was lost in this way; each levulose tolerance test from first to last occupied four hours and a half, and this is a point of some importance which will be referred to at a later stage when a criticism of the test from the point of view of its practicability is undertaken.

BIOCHEMICAL METHODS.

After a preliminary series of blood sugar analyses by Cole's and Maclean's microchemical method using both blood and glucose solutions of known concentrations it was found that Maclean's method was both more accurate and quicker.

Cole's method gave a constant error of 10-15% below the correct sugar content, and the variation of 5% between duplicates - due, no doubt, to an extra pipette measurement. Maclean's method, on the other hand, gave an error of less than 5%, and a variation between duplicate specimens of about 1% - both of these results being well within the limit of accuracy necessary in the levulose test. It must be understood that these remarks apply to the methods in the hands of the writer, and doubtless a skilled chemist would show results with a very much smaller margin of error.

If duplicate specimens showed a greater difference than 0.010% of glucose the curve was interpreted with suspicion and caution.

METHOD OF COLLECTION OF THE BLOOD.

The differences between the sugar content of venous and capillary blood have been shown to be negligible (Forster, Journal of Biological Chemistry 55: 291-301: February 1923), both methods of collection/

collection were employed and the charts which were obtained were considered to be comparable. A point of some practical importance is the difficulty in some cases of obtaining blood by means of puncturing the finger as is recommended by Maclean - the blood may clot in the pipette if there is any delay in its collection owing to a diminished rate of flow, etc., and it may be necessary to make several pricks in order to obtain the requisite 0.2cc. which is a fairly large drop. In such cases venipuncture was usually performed and the blood oxalated with glucose-free calcium oxalate, the measurement of 0.2cc. from the test-tube containing the blood was an easy matter. It is a debatable question whether more pain is caused by venipuncture with a very sharp fine needle than by stabbing the pulp of the finger with a sufficiently broad instrument to assure a plentiful flow of blood.

#### BLOOD SUGAR IN PREGNANCY, LABOUR AND LACTATION.

The findings of the writer will be given here as it was considered necessary to ascertain if there was any difference between the blood sugar percentage in normal adults and in pregnancy, labour, and the puerperium. It is essential to know that the curves obtained in these conditions are comparable to those obtained by Spence and Brett in the non-pregnant state.

TABLE I.FASTING BLOOD SUGAR DURING NORMAL PREGNANCY.

<u>Case</u>	<u>Blood sugar Percentage</u>	<u>Para.</u>	<u>Duration of Pregnancy</u>	<u>Comments</u>
1.	0.090%	0	32 weeks	Vaginal discharge
2.	0.093%	0	32 weeks	Normal
3.	0.098%	4	Term	Normal
4.	0.100%	0	Term	Normal
5.	0.080%	0	Term	Normal
6.	0.093%	0	Term	Normal
7.	0.093%	0	Term	Normal
8.	0.100%	4	Term	Normal
9.	0.075%	0	Term	Normal
10.	0.093%	0	Term	Normal
11.	0.100%	0	Term	Normal
12.	0.100%	12	Term	Normal
13.	0.093%	0	Term	Normal
14.	0.093%	0	Term	Normal

Average Blood Percentage = 0.093%.



TABLE II.FASTING BLOOD SUGAR DURING ABNORMAL PREGNANCY.

<u>Case</u>	<u>Blood sugar Percentage</u>	<u>Para.</u>	<u>Duration of Pregnancy</u>	<u>Comments</u>
1.	0.117%	3	6½ months	? Toxaemia - vomiting.
2.	0.114%	0	8½ months	Eclampsia
3.	0.110%	1	Term	Chorea Gravidarum
4.	0.075%	0	Term	Nephritic Tox. (2 day's fasting)
5.	0.075%	0	Term	Pre-eclamptic Tox. (2 day's fasting)
6.	0.075%	1	6½ months	Mitral Stenosis, con- gestion of liver.
7.	0.080%	0	Term	Nephritic Tox. (fasting 2 days).

TABLE III.BLOOD SUGAR DURING THE PUERPERIUM.

<u>Case</u>	<u>Blood sugar Percentage</u>	<u>Day of Puerperium</u>	<u>Comments.</u>
1.	0.096%	4th	
2.	0.114%	4th	
3.	0.109%	2nd	
4.	0.093	9th	
5.	0.102%	9th	
6.	0.100%	3rd	
7.	0.084	1st	
8.	0.080%	3rd	
9.	0.084%	7th	
10.	0.080%	2nd	

Average Blood Sugar Percentage = 0.094%.

(These specimens were withdrawn immediately before the mother suckled the child, in order to obtain a resting as well as a fasting blood sugar).

TABLE IV.

BLOOD SUGAR DURING AN ABNORMAL PUERPERIUM.

In a case of slight jaundice and vomiting which developed on the second day of the puerperium to clear up completely in a few days, the fasting blood sugar on the third day after delivery was 0.146%.

TABLE V.

BLOOD SUGAR IMMEDIATELY AFTER LABOUR.

- Case 1. 1-para. Duration of labour - 7 hours.  
Delivery spontaneous. Twilight sleep with terminal light chloroform anaesthesia.  
Blood sugar percentage = 0.087%.
- Case 2. 1-para. Duration of labour - 15 hours.  
Delivery spontaneous. Blood sugar percentage = 0.110%.
- Case 3. 1-para. Duration of labour - ? Spontaneous delivery. Twilight sleep - the patient was very noisy. Blood sugar percentage 0.142%.

Case 4./

- Case 4. 1-para. Duration of labour -  $37\frac{1}{2}$  hours.  
Delivery spontaneous. Twilight sleep,  
terminal chloroform anaesthesia, the patient  
was extremely noisy and restless. Blood  
sugar percentage = 0.132%.
- Case 5. 2-para. Duration of labour - 29 hours.  
Spontaneous delivery. The labour was very  
easy. Blood sugar percentage = 0.102%.
- Case 6. 4-para. Duration of labour - ?  
Spontaneous delivery. The second stage was  
protracted and exhausting. Blood sugar  
percentage = 0.155%.
- Case 7. 1-para. Duration of labour - 18 hours. The  
breech became impacted and necessitated  
extraction under chloroform anaesthesia,  
an exhausting labour. Blood sugar per-  
centage = 0.137%.
-

CONCLUSIONS FROM THESE TABLES.

Although the series is much too small to draw any very definite conclusions as regards the fasting blood sugar percentage in the conditions enumerated above, it would appear that the blood sugar during pregnancy and lactation is within normal limits. In the disorders of pregnancy the evidence was rather conflicting, whilst immediately after the termination of labour the blood sugar seemed to be raised in proportion to the severity of the labour, which is to be expected. The writer felt justified in pursuing the study of levulose tolerance during the course of pregnancy and the puerperium.

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THE LEVULOSE TOLERANCE TEST -  
ABSTRACT OF CASES WITH CHARTS.

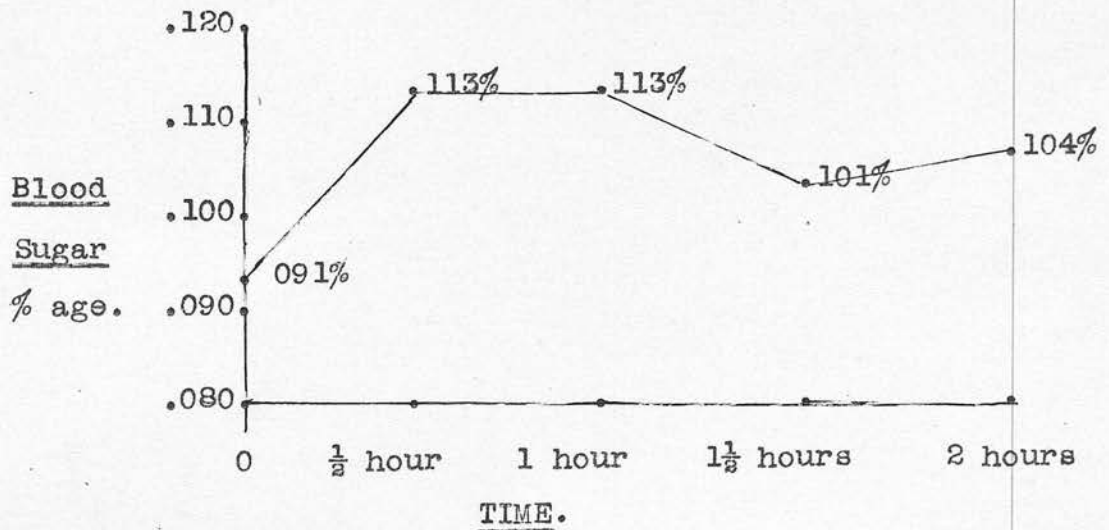
Case 1. Mrs Slater. aet. 47 years. 8-para.

8 months pregnant. Hydramnios.

Present pregnancy. There was excessive vomiting in the early months. She was admitted to hospital on account of hydramnios with slight dimness of vision, and slight oedema of the hands and feet. At the time of the test these last symptoms had cleared up. Examination revealed ~~no~~ signs of slight myocardial weakness. B.P. 130/90.

Urine - no abnormal constituents.

Blood Sugar Curve after Levulose.



There is thus a rise of 0.022% - a very doubtful positive result.



Case 2. Mrs Carmichael. aet. 24 years. 1-para.

Full-time. Toxaemia.

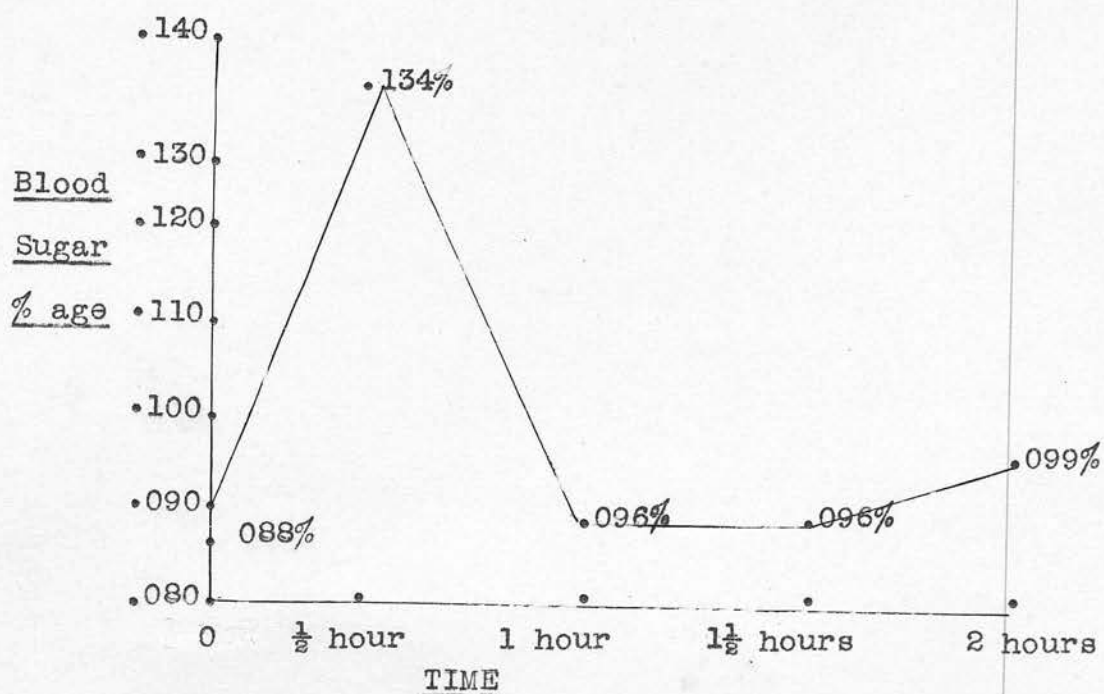
Present Pregnancy. The patient had rather excessive vomiting during the early months, but this subsided. During the past week she has had headaches, dimness of vision, oedema of the legs and hands, and pain under the right costal margin - for these symptoms she was sent to hospital.

Examination revealed little of note apart from the oedema. B.P. 140/100.

Urine - no abnormal constituents.

There were therefore the symptoms of a slight toxaemia, the interesting point being the constant absence of albumen. The subsequent history of the pregnancy was uneventful and the labour was normal.

Blood Sugar Curve after Levulose.



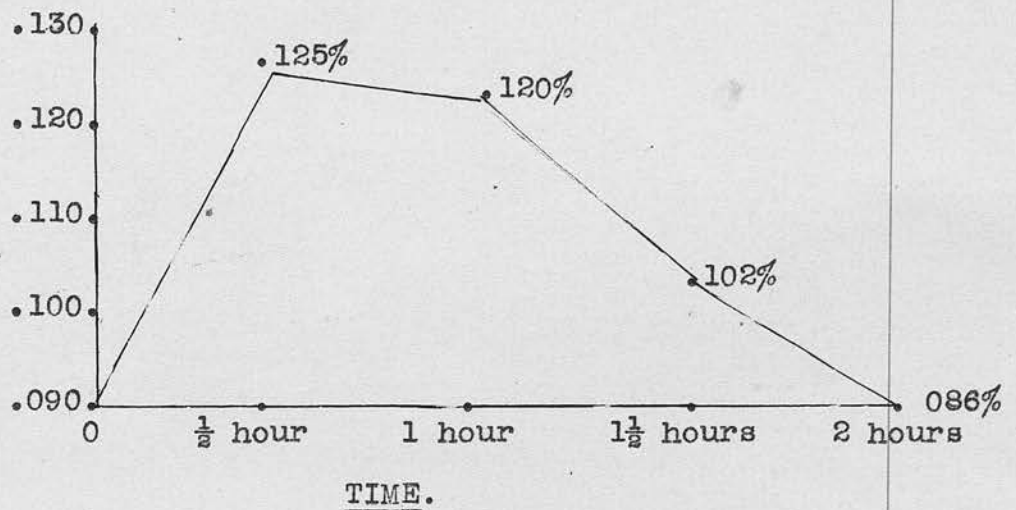
There is thus a rise of 0.046% - a definitely positive result.

Case 3. Catherine Regan. aet. 22 years. 0-para.  
8 months. Normal.

Present pregnancy. The pregnancy was quite normal,  
and the patient was in hospital for the treatment of  
a vaginal discharge.

Urine. No abnormal constituents.

Blood Sugar Curve after Levulose.



The curve showed a rise of 0.035%, which is above the limit of normality - why this should be was not explained either at the time or by the subsequent progress of the case which was uneventful.

-----

Case 4. Mrs Fraser. aet. 40 years. O-para.

8½ months. Eclampsia.

Present pregnancy. No history was available, and the patient was sent into hospital with the diagnosis of eclampsia as she had had one fit with generalised oedema, and massive albuminuria.

A levulose tolerance test was started, but the patient vomited the sugar within a few minutes of its administration. The test was therefore not continued.

Initial blood sugar = .114%.

Case 5. Mrs Brookes. aet. 35 years. O-para.

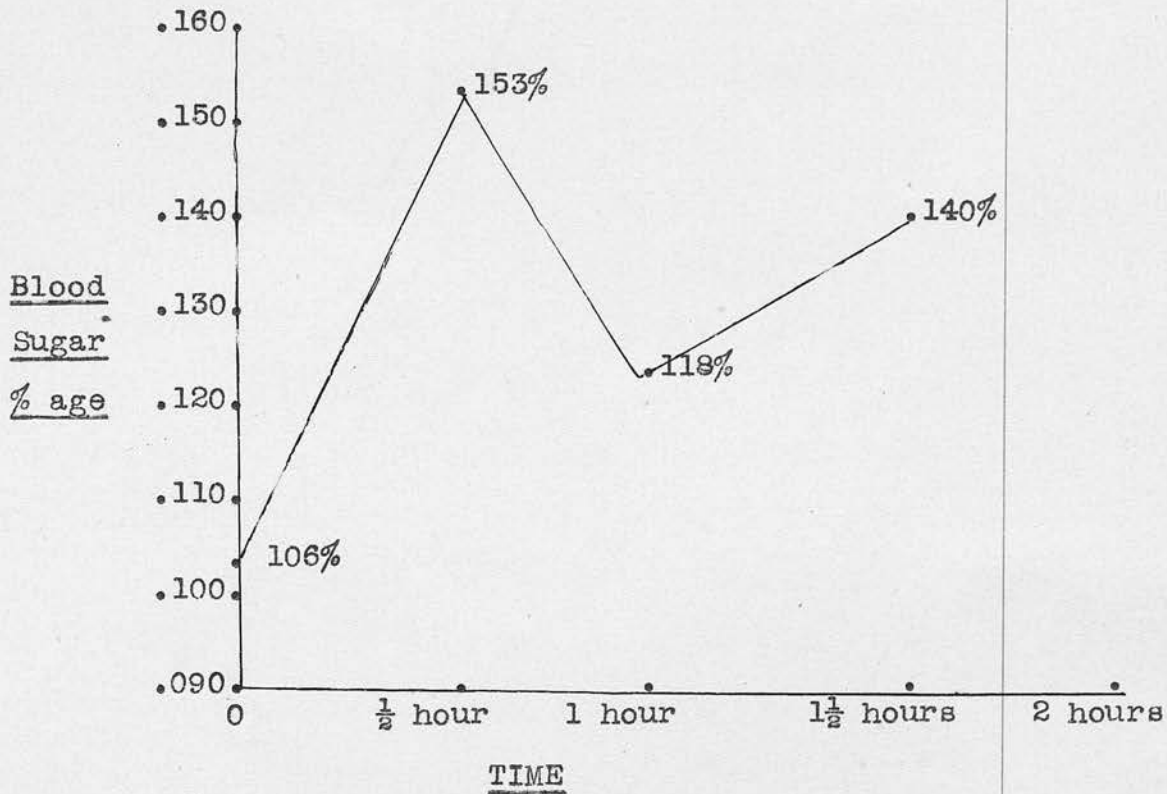
Abortion at 2½ months.

Present pregnancy. The patient had an abortion at the end of 10 weeks of pregnancy - apart from this occurrence the pregnancy had been free from abnormal symptoms.

Examination prior to the performance of the test (5 days after the abortion) revealed no abnormalities, and the puerperium was afebrile.

Urine - no abnormal constituents.

Blood/

Blood Sugar Curve after Glucose.

This marked rise was apparently due to psychical causes - the secondary rise occurring just after the patient had passed a clot of blood per vaginam, she was greatly alarmed and required very soothing treatment before her fears were allayed.

It is to illustrate the effect of extraneous causes upon the sugar curve that this chart has been included.

Case 6. Mrs Hood. aet. 38 years. 3-para.

6½ months. ? Toxaemia.

Present pregnancy. The pregnancy had been uneventful, until four days before admission when she had a rigor and diarrhoea - on the three successive days she was quite well; the day before admission she felt faint, went to bed and began to vomit, the vomiting continued until she was admitted. There was some pain in the right loin.

Examination. The heart and lungs were normal, the abdomen was normal except for a distended ascending colon which was the seat of pain, but not of tenderness. She was very constipated.

Urine - albumen, acetone, and indol were present.

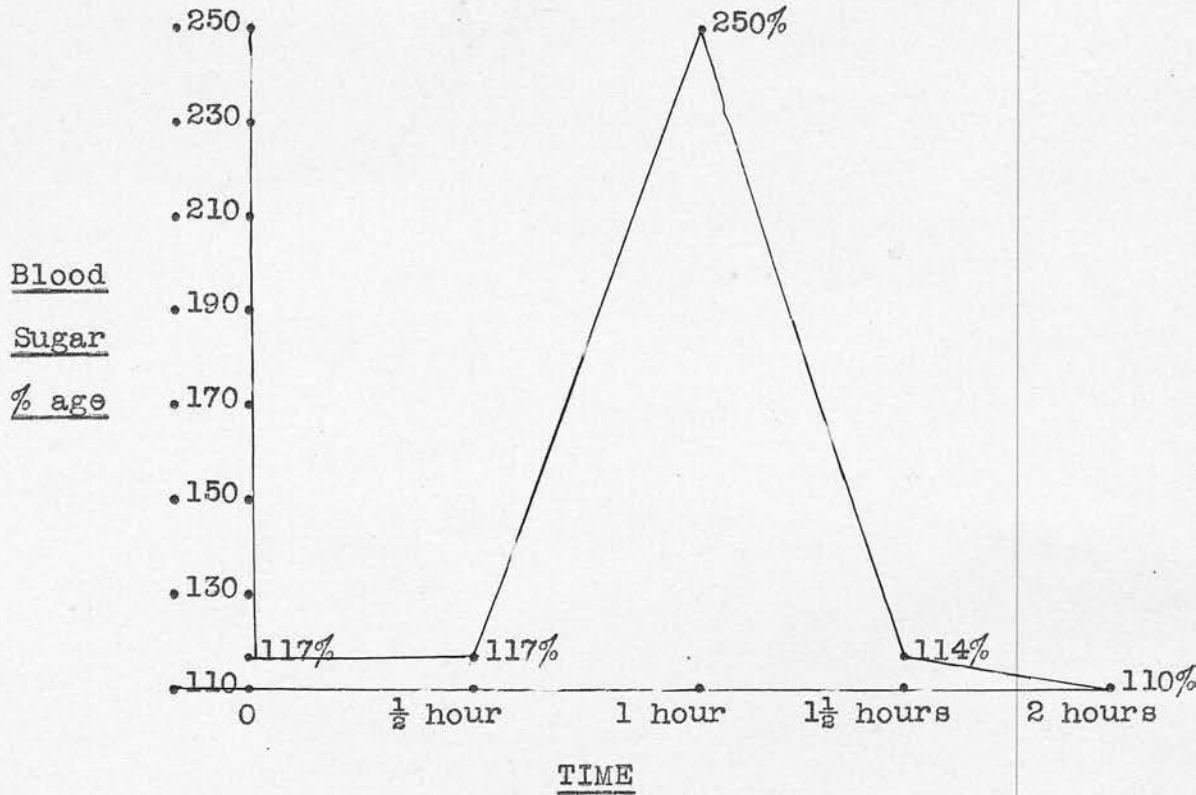
Provisional diagnosis. Constipation and colic.

? toxaemia.

Treatment. Gastric and colon lavage. Starvation for 24 hours. The vomiting and other symptoms cleared up in three days and the patient was discharged after a stay of 9 days. A levulose test was done the day before discharge, i.e. 5 days after the symptoms had subsided.

BLOOD/



BLOOD SUGAR CURVE AFTER LEVULOSE.

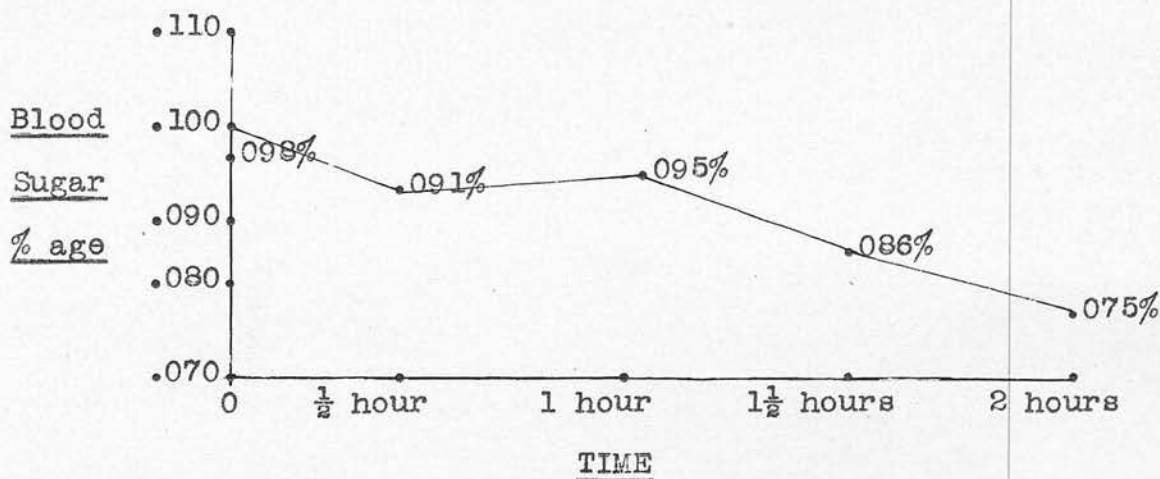
This anomalous curve was not explained, it certainly did not reflect the clinical condition of the patient, who was almost fit again.

-----

Case 7. Mrs Bennet. 4-para. aet. 32 years. Term.  
Normal pregnancy.

History. This was a normal pregnancy, the urine contained no abnormal constituents, and the blood pressure was within normal limits.

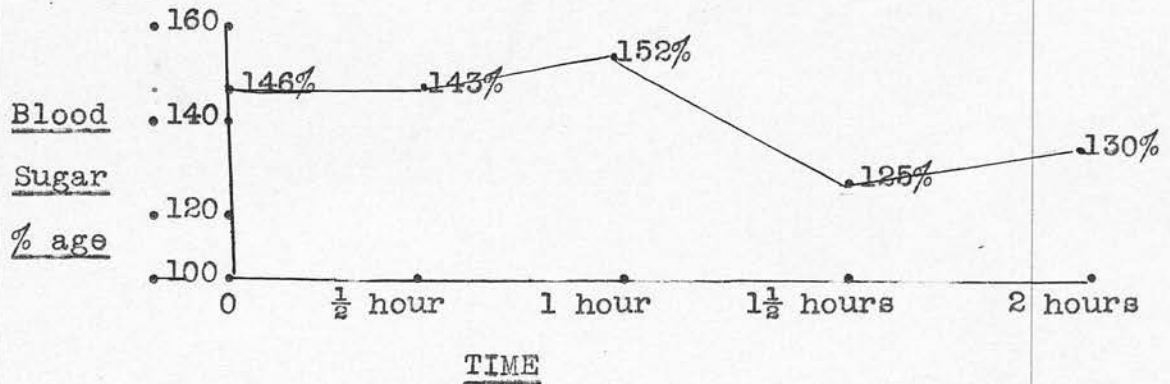
BLOOD SUGAR CURVE AFTER LEVULOSE.



Case 8. Mrs Duncan. aet. 26 years. 1-para. 3rd  
 day puerperium. Jaundice.

History. After the termination the patient developed slight jaundice and hepatic pain. These symptoms cleared up in a few days without a definite diagnosis. The test was made on the third day of the puerperium when the jaundice was at its height.

BLOOD/

BLOOD SUGAR CURVE AFTER LEVULOSE.

The interpretation of this curve was rather difficult owing to the abnormal height of the initial blood sugar, there was not however a rise of any note after the levulose as one would have expected in the presence of jaundice, although it was only of slight degree - it may be that it was an obstructive jaundice without hepatic derangement.

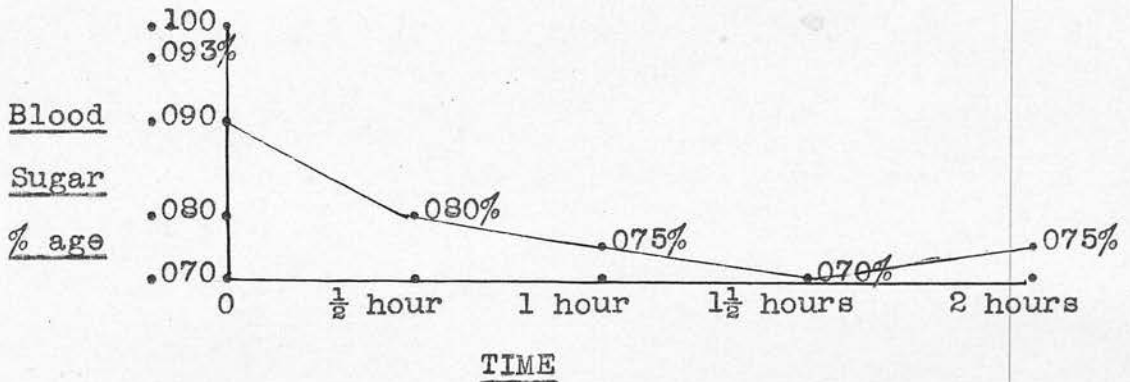
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Case 9. Mary Klein. aet. 20 years. 0-para.

8½ months. Normal pregnancy.

This was an absolutely normal pregnancy, the urine and the blood pressure showed no abnormality.

BLOOD SUGAR CURVE AFTER LEVULOSE.



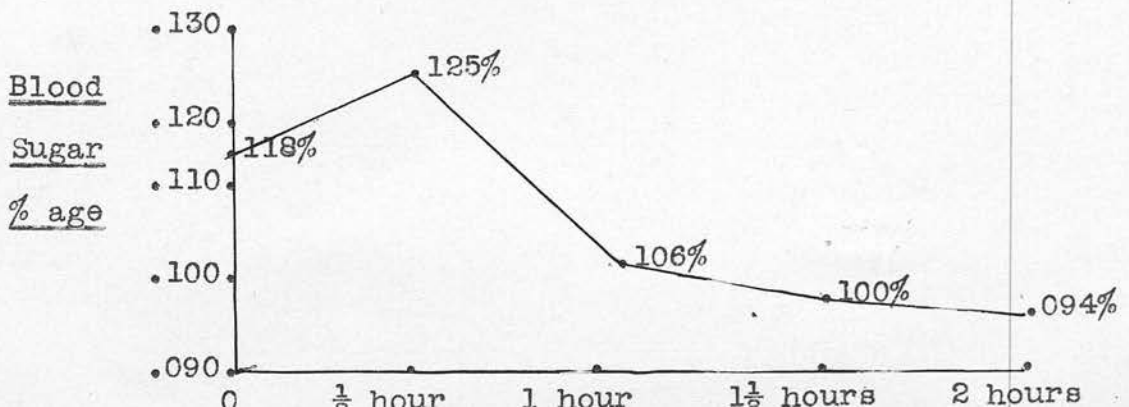
Case 10. Mrs Blackwood. aet. 23 years. 1-para.

6½ months. Chorea.

History. The patient had had chorea in childhood.

She had a recrudescence of the disease in a very mild form which started a week before admission. There were no signs or symptoms of toxæmia, and the chorea cleared up completely in the course of a few weeks.

BLOOD SUGAR CURVE AFTER LEVULOSE.



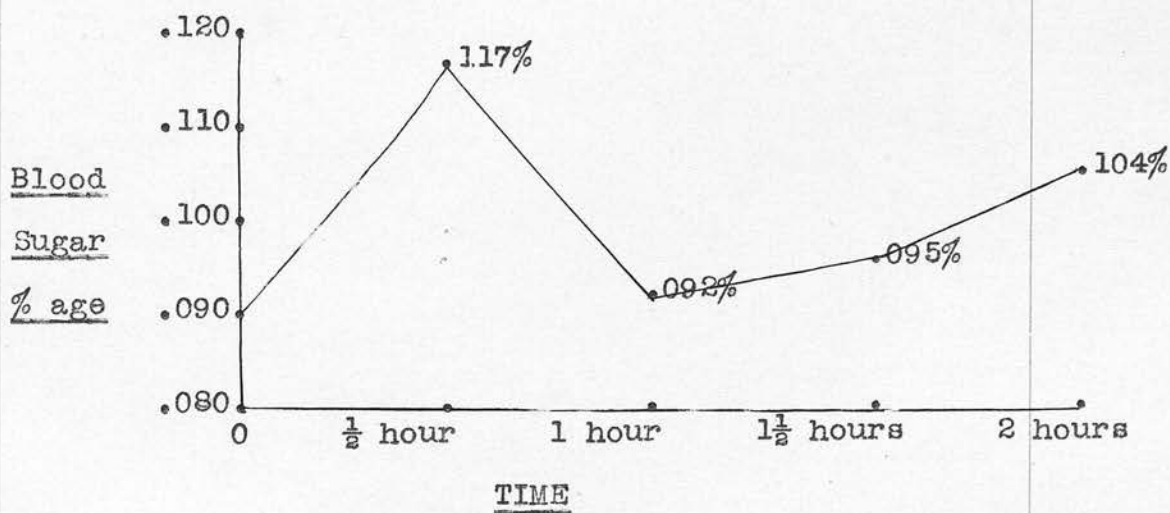
Case 11. Mrs McDermott. aet. 19 years. 1-para.

Term. Chorea.

History. The patient suffered from chorea since childhood, attacks occurring annually; during pregnancy she began to have movements from the first and they have continued till term. The movements were bilateral and fairly severe and continuous.\* After delivery they diminished, but were still present to some extent.

Urine - normal. B.P. 130/90.

BLOOD SUGAR CURVE AFTER LEVULOSE.



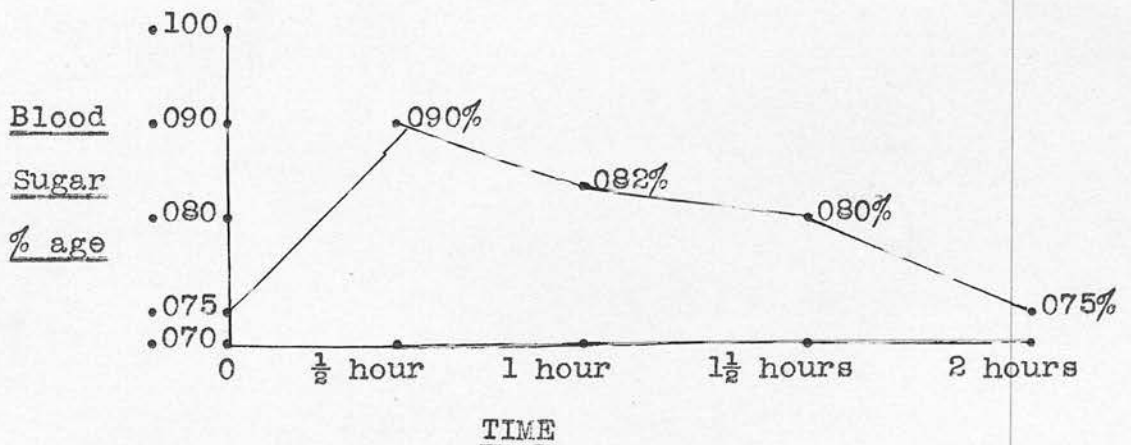


Case 12. Margt. Smith. aet. 22 years. 9-para.

Term. Nephritic Toxaemia.

History. The patient had been quite well until a week before admission when she developed blurring of vision, and oedema of the feet. At the time of the test the blood pressure was 170/136, and the urine contained 0.4% of albumen as estimated by Esbach's albuminometer. She was being treated by starvation and free stimulation of the eliminative functions.

BLOOD SUGAR CURVE AFTER LEVULOSE.



The rise of 0.015% may be regarded as within normal limits.

Case 13. Mary Sweeney. aet. 28 years. 0-para.

Term. Pre-eclamptic  
Toxaemia - mild.

History. The patient was quite well until two weeks before admission when she developed oedema of the feet, dimness of vision, failure of appetite, and cough. A few days later the oedema spread to the hands and face, and she became rather dyspnoeic.

Examination. B.P. 180/154.

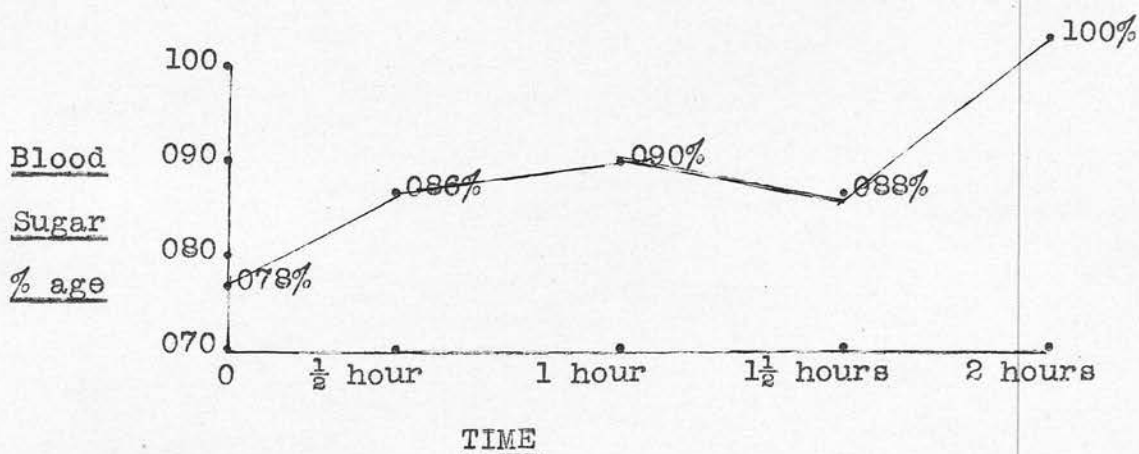
Urine - albumen strongly positive reaction. This was at the time of the test.

Progress. Four days later she was delivered spontaneously, the placenta was markedly infarcted.

Puerperium normal.

Treatment was by means of starvation and free elimination.

BLOOD SUGAR CURVE AFTER LEVULOSE.

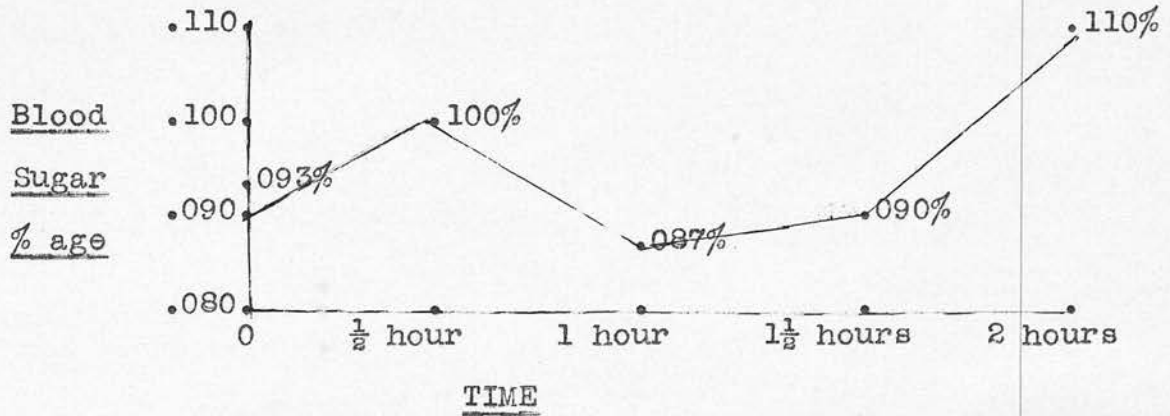


The curve is abnormal and continues to rise up to the second hour - this was interpreted as signifying a mild liver impairment.

Case 14. Mrs Walker. aet. 38 years. 1-para.

Term. Normal pregnancy.

BLOOD SUGAR CURVE AFTER LEVULOSE.



This curve - a rise of 0.013% at the end - was normal.

Case 15. Mrs Arnall. aet. 25 years. 1-para.

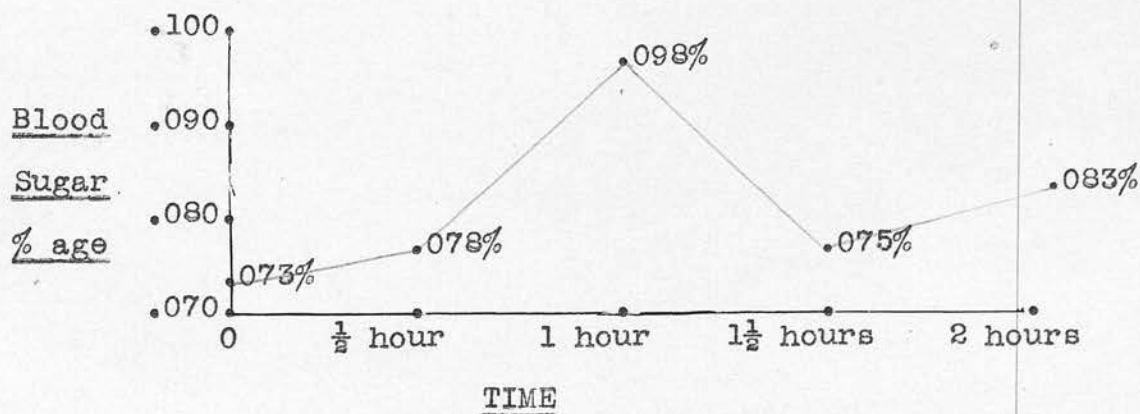
7 months. Mitral Stenosis.

History. The patient had mitral stenosis, and the symptoms were as usual greatly aggravated by her pregnancy. She was admitted to hospital with dyspnoea, bronchitis, and failure of cardiac compensation.

Examination. Mitral stenosis and incompetence, crepitations at both bases. The urine did not contain albumen.

Treatment. Rest in bed, Caesarean section at term, and sterilisation.

BLOOD SUGAR CURVE AFTER LEVULOSE.



This curve shows a rise at the end of an hour of .025% which is significant of a slight liver impairment. This is quite in keeping with the clinical data.

To summarise the cases:-

(1)	Mild pre-eclamptic toxæmia.	Rise of .022%.
(2)	Definite " "	Rise of .040%.
(3)	Normal Case.	Rise of .035%.
(4)	Eclampsia.	Patient vomited the sugar.
(5)	Glucose tolerance test.	Marked rise from psychic causes.
(6)	Very doubtful toxæmia.	Rise of .133%.
(7)	Puerperal jaundice.	Normal curve.
(8)	Normal Case.	Normal curve.
(9)	Chorea Gravidarum.	Rise of .007%.
(10)	" "	Rise of .007%.
(11)	Nephritic toxæmia.	Rise of .015%.
(12)	Pre-eclamptic toxæmia.	Rise of .022%.
(13)	Normal case.	Rise of .013%.
(14)	Mitral stenosis with bad compensation.	Rise of .025%.

-----



CONCLUSIONS.

It will thus be seen that the tests gave results in these cases which, with the exception of No.6, were quite in keeping with the clinical findings. In many of the conditions which were under treatment at this time an endeavour was made to carry out a levulose tolerance test, but after a variable time the patients rejected the sugar and the test could not be completed. Such cases were hyperaemesis gravidarum, some pre-eclamptic toxæmias, one eclamptic case (the only one who was sufficiently conscious to swallow the sugar). Unless it is considered necessary or desirable to pass a stomach tube for the purpose of administering the sugar the test will be found to be inapplicable in the vast majority of the cases in which a knowledge of the functional capacity of the liver is of importance as a guide to treatment and to prognosis.

The time necessary for the performance of the test and for the estimation of the ten blood sugars is an objection which must be considered.

The view which the writer takes is that the levulose tolerance test has a very limited sphere of usefulness in the study of the toxæmias of pregnancy, as it is inapplicable to the very cases in which it is important.

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THE BLOOD DIASTASE TEST IN PREGNANCY.

In a series of six cases of normal and abnormal pregnancies the blood diastase was estimated by the method of Fyfe (see section on Blood and Urinary Diastase), as the series is so small it is impossible to draw any conclusions from the results, but they are included in tabular form.

Case	Blood Sugar	Starch Prepn.	Diastase.	Para.	Time	Remarks.
1	.100%	.250%	15	0	Term	Normal
2	.080%	.156%	7.6	0	Term	Nephritic Tox.
3	.093%	.212%	11.7	0	Term	Normal
4	.093%	.156%	6.3	0	Term	Normal
5	.100%	.187%	8.7	12	Term	Normal
6	.075%	.100%	2.5	0	Term	Pre-eclamptic toxaemia.

ROCH'S METHYLENE BLUE TEST.

ROCH'S METHYLENE BLUE TEST.

A series of 56 cases of various diseases in children was submitted to this test, the patients were upon the usual hospital diet, at 8 a.m. each received 0.002 gramme of methylene blue (Burroughs, Wellcome) dissolved in one drachm of distilled water, and the urine secreted between the hours of 12 noon and 4 p.m. was collected for examination. The development of green colour was quite definite and required no special means for its detection.

The results are as follows:-

POSITIVE RESULTS./



POSITIVE RESULTS.

Name	Sex	Age	Disease	Result.
1. Hext.	F.	14	Chorea, convalescent, Ht. n.	Faint.
2. Perrott.	F.	13	ditto	Definite.
3. Easely.	F.	8	ditto	Definite.
4. Lunn.	M.	14	ditto	Faint.
5. Dixon.	M.	13	Aortic stenosis and Incomp. Compensation poor.	Definite.
6. Long.	M.	9	Mitral incompet. Liver normal.	Faint.
7. Titteral.	M.	12	Mitral sten. & inc. Liver normal.	Definite.
8. Walker.	M.	14	Chronic otorrhoea.	Faint.
9. Warnock.	M.	7	ditto. Recovering Influenza	Very marked.
10. Stewart.	M.	12	Mitral stenosis. Liver Normal.	Definite.
11. Gunther.	M.	12	Chronic otorrhoea.	Definite.
12. May.	M.	12	Conval. ac. rheum- atism. Heart and Liver normal.	Definite.
13. Green.	M.	7	Bronchitis.	Definite.

POSITIVE RESULTS. (continued).

Name	Sex	Age	Disease	Result.
14. Taylor	M.	10	Conv. encephalitis lethargica.	Definite.
15. Milton.	M.	9	Patient I-V septum. Liver normal.	Marked.
16. Grizzell	M.	6	Active endocarditis (aortic)	Definite.
17. Gillings	M.	7	Otorrhoea. Liver normal.	Marked.
18. Hayes.	M.	4	Acute endocarditis (mitral)	Extremely marked.
19. Divine.	M.	5.	Otorrhoea. Liver normal.	Definite.
20. Parks.	M.	6	ditto	Marked.
21. Gould.	M.	7	Mild secondary anaemia. Liver normal.	Marked.
22. Nugent.	M.	7.	Pulmonary fibrosis - pneumonic.	Faint.
23. Blackmore	M.	11.	Otorrhoea. Liver normal.	Marked.
24. Lanark.	M.	11.	Pulmonary fibrosis - pneumonic.	Faint.
25. Holt.	F.	13.	Mitral incomp. hyper- trophy of heart. Liver not enlarged.	Faint.

NEGATIVE RESULTS.

Name	Sex.	Age	Disease	Result.
26. Butlin.	M.	13	Conval. chorea, heart and liver normal.	Negative.
27. Bridgman.	F.	9	ditto	Negative.
28. Harris.	F.	8.	Early mitral incomp. Liver normal.	Negative.
29. Newman.	F.	9.	Conval. ac. rheum. Heart & liver normal.	Negative.
30. Oates.	F.	9.	Chronic otorrhoea.	Negative.
31. Alexander.	F.	9.	ditto.	Negative.
32. Jones.	F.	8.	Mitral incomp. slight.	Negative.
33. Biggs.	F.	7.	Bronchitis - chronic.	Negative.
34. Downs.	F.	9.	Conval. chorea. Heart normal.	Negative.
35. Hogan.	F.	15.	ditto	Negative.
36. Millsome.	F.	8.	Acute chorea. Heart Normal.	Negative.
37. Payne.	F.	8.	Chronic otorrhoea.	Negative.
38. Edwards.	M.	13.	Aortic sten. & incomp. Liver Normal.	Negative.
39. Sadler.	M.	13.	Aortic sten. & incomp. chr. inter. neph. Liver normal.	Negative.
40. Seymour.	M.	15.	Aortic sten. & incomp. liver normal.	Negative.
41. Diggins.	M.	13.	Pick's disease. Liver four fingers below costal margin. syphilitic.	Negative.

NEGATIVE RESULTS (continued).

Name	Sex	Age	Disease	Result
42. Stanton.	M.	10	Otorrhoea.	Negative.
43. Holland.	M.	12	ditto.	Negative.
44. Dorman.	M.	8	ditto.	Negative.
45. Lovell.	M.	8	Conval. ac. rheum. Liver normal.	Negative.
46. Pitcher.	M.	8	ditto.	Negative.
47. Clark.	M.	11	Posthaemorrhagic secondary anaemia.	Negative.
48. Ward.	M.	8	Conval. chorea.	Negative.
49. Green.	M.	7	Otorrhoea	Negative.
50. Fishlock.	M.	13	ditto	Negative.
51. Hume.	M.	14	Conval. ac. rheum. mitral incomp.	Negative.
52. Rider.	M.	8	Conval. chorea.	Negative.
53. Tilliard.	M.	13	Chronic otorrhoea.	Negative.
54. Singleton.	M.	9	Conval. chorea.	Negative.
55. Bloom.	M.	11	Conval. ac. rheum.	Negative.

The terms faint, definite, marked, very marked, and extremely marked indicate the varying degrees of concentration of the dye in the urine. Liver normal means that the organ was not enlarged either on palpation or percussion. The presence of the dye in the urine as a colourless chromogen was not sought. Roch's series showed that this precaution was unnecessary.

An analysis of the details given in these tables furnishes/



furnishes the following data:-

(1) The cases showing a positive reaction.

(a) The average was ----- 10 years.

The oldest patient was ----- 14 years.

The youngest patient was ----- 4 years.

(b) The average age of the patients grouped according to the intensity of the reaction.

Faint reaction ----- 13 years (5 cases).

Definite reaction ----- 10 years (11 cases).

Marked reaction ----- 8 years (5 cases).

Very marked reaction ----- 7 years (1 case).

Extremely marked reaction - 4 years (1 case).

(c) The number of patients of 10 years or over was 52%.

(2) The cases showing a negative reaction.

(a) The average age was ----- 10 years.

The oldest patient was ----- 15 years.

The youngest patient was ----- 7 years.

(b) The number of patients of 10 years or over was 10%.

(3) The relation of positive and negative reactions.

Of the total cases 45% gave a positive reaction, and if those cases under the age of 10 years are excluded from the series this percentage is increased to 50%.



(4) Discussion.

In the series there were only two patients in whom any abnormality in the excretion of methylene blue was anticipated on clinical grounds - one was a case of severe azotaemic nephritis whose kidneys were probably too badly damaged to excrete the dye (Sadler, No.39, negative). The other was a polyserositis with presumably a hypertrophic hepatic cirrhosis of syphilitic origin whose liver was able - according to Roch's views - to fix and remove the dye from the blood stream (Diggins, No.41, negative). In this case a positive reaction was confidently awaited.

It may be objected that the age of the patients has an influence on the result of the test, as the dose of the dye was <sup>not</sup> adjusted in relation to the weight or the age; this objection is not supported by an analysis of the average age of the positive and negative groups which was the same in each case, but it must be admitted that in the positive cases the degree of intensity of the reaction increased in inverse ratio to the age of the patients. To overcome this possible objection the cases under the age of 10 years were excluded, this made little difference to the incidence of positive findings, merely increasing the percentage from 45 to 50.

It must be concluded, therefore, that this test is/

is without value in the elucidation of the functional capacity of the liver in childhood, as 45% to 50% of cases with presumably normal livers give positive results.

In normal adults other workers have found an even greater percentage of positive results.

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THE SODIUM SALICYLATE TEST.

TECHNIQUE.

At 6 a.m. the patients had the usual hospital breakfast of tea, bread and butter; at 7 a.m.  $\frac{1}{2}$  grain of sodium salicylate in aqueous solution was administered. A specimen of urine was collected at 9 a.m. for use as a control, and the urine secreted between the hours of 9 a.m. and 11 a.m. was used for the actual test.

The urine was first made frankly acid by the addition of two or three drops of strong nitric acid to the total amount in the urine glass. A test-tube was filled with an aqueous solution of ferric perchloride similar in tint to the urine (usually this meant a strength of 1-2%), and by means of a test tube of gross calibre the urine was dropped in. A negative reaction was one in which no colour changes took place on the interface between the urine and the perchloride, a positive reaction was indicated by the development of a violet colour of varying intensity round the drop of urine.

The control was similarly tested with the perchloride solution to exclude the presence of substances such as diacetic acid which might invalidate the results.

The colour changes in the positive cases were easy to observe, a very faint reaction could be checked by reading before a dark surface.

The/

The test was carried out in 33 cases of various medical and surgical diseases in children, and in 14 cases the effect of operation with its accompanying anaesthesia was investigated. In a certain number of cases the presence of urobilin by Grimbert's method, that of bile salts by Hay's method, and of bilirubin by Grimbert's method was tested; the results are tabulated below.

POSITIVE RESULTS.

No.	Name	Sex	Age	Disease	Remarks
1.	Diggins	M.	14	Pick's Disease. Liver enlarged 4 fingers below costal margin.	Hay ++ Uro & Bil.- Test strongly posi- tive.
2.	Dixon	M.	14	Mitral sten. & incomp. Liver normal.	Hay, uro and bil.
3.	Sadler	M.	13	Aortic st. & incomp. liver norm. Inter neph.	Hay, uro and bil.
4.	Wooster.	M.	15	Aortic inc. Liver normal.	Hay & uro + bil.
5.	Flemming	M.	15	Subac. parench. Nephritis	Hay & bil + Uro.
6.	Simmonds	M.	7	Chr. Otorrhoea.	Very faint. Hay, Uro, bil.-
7.	Thaxter.	M.	6	Ulcerative stomatitis.	Very faint. Hay, Uro, bil.-
8.	Abrahams.	M.	15	Chr. Otorrhoea.	Hay & bil. - Uro. +
9.	Adams.	M.	12	ditto	Hay, bil, uro -
10.	Carpenter.	M.	14	ditto	ditto
11.	Tibber.	F.	12	ditto	Hay +
12.	Scurr.	M.	9	ditto	Very faint. Hay -



NEGATIVE RESULTS.

No.	Name	Sex	Age	Disease	Remarks
13.	Baars	M.	15	Conval. ac. rheum. Heart and liver normal.	Hay, uro, bil -
14.	Long	M.	9	Early mitral stenosis. Liver enlarged one finger.	ditto
15.	Smith	M.	12	Patent foramen ovale, very cyanosed. ?Riedel's lobe.	Hay + uro and bil -
16.	Price	M.	15	Chr. Otorrhoea	Hay, uro, bil -
17.	Highlands.	M.	13	ditto	ditto
18.	Keens.	M.	14	Conval. ac. rheum. slight mitral incompetence.	ditto
19.	Stewart.	M.	12	Mitral stenosis. Liver normal.	ditto
20.	Bosley.	M.	9	Conval. Chorea.	ditto.
21.	Hampton.	M.	4	Ac. simple endocarditis (rheum). Liver nor en- larged.- (mitral).	Hay, uro, bil -
22.	Grizell.	M.	6	Ac. simple endocarditis. (Aortic) Liver not en- larged).	ditto
23.	Holmes.	M.	10	Chr. Otorrhoea.	Urobilin +
24.	Rayner.	M.	13	ditto	Uro, Hay, bil -
25.	Knight.	M.	3	Scald on chest - healing.	ditto
26.	Cole.	M.	12	Chr. Otorrhoea.	ditto
27.	Arter.	M.	11	ditto	ditto
28.	Holloway.	M.	8	ditto	ditto
29.	Gadson.	M.	9	ditto	ditto
30.	Rogers.	M.	10	ditto	ditto
31.	Randle.	M.	9	ditto	ditto
32.	Butcher.	M.	11	ditto	ditto
33.	Giddons.	M.	6	Mitral incompetence, liver normal.	ditto

THE EFFECT ON ANAESTHESIA ON THE TEST.

In a small series of cases the salicylate test was performed both before and after radical mastoid operations on the ear; the cases were all normal apart from the otorrhoea, and it is not necessary to give full details of them from this point of view. A salicylate test was done on the morning of operation, the operation was performed in the afternoon, and the following morning a further test was carried out. Although the results were in most cases quite negative they will be tabulated for the sake of comparison with the results obtained by other workers with different tests, such as the test of glycuronuria

TABLE /

No.	Name.	Age.	Sex.	Anaesthetic.	Before Operation		After Operation	
					Salic.	Hay.	Salic.	Hay.
1.	Holmes	10	M.	Eth. chl. & ether.	Neg.	Neg.	Neg.	Neg.
2.	Rayner	13	M.	C E 2 3	Neg.	Neg.	Neg.	Neg.
3.	Abrahams.	15	M.	C E 2 3	Ft.	Pos.	Pos.	Pos.
4.	Adams.	12	M.	C E 2 3	V.Ft.	Neg.	Neg.	Neg.
5.	Scurr.	8	M.	C E 2 3	V.Ft.	Neg.	Neg.	Neg.
6.	Carpenter.	14	M.	Ether.	V.Ft.	Neg.	Neg.	Pos.
7.	Knight.	3	M.	Ether.	Neg.	Neg.	Pos.	Pos.
8.	Cole.	12	M.	C E 2 3	Neg.	Neg.	Neg.	Neg.
9.	Tibber.	12	F.	Ether.	Pos.	Pos.	Neg.	Neg.
10.	Arter.	11	M.	Ether.	Neg.	Neg.	Neg.	Neg.
11.	Holloway.	8	M.	Ether.	Neg.	Neg.	Neg.	Neg.
12.	Gadson.	9	M.	Ether.	Neg.	V.Ft.	Neg.	Neg.
13.	Randle.	9	M.	Ether.	Neg.	Neg.	Neg.	Neg.
14.	Butcher.	11	M.	C E 2 3	Neg.	Neg.	Neg.	Neg.

The average duration of the operations was between one hour and one hour and a half. In Case 7 the operation was a skin-graft for a large scald on the chest, but this is the only instance in which the conditions were different, as regards the operation and its duration.

CONCLUSIONS.

Of the positive results there was only one case (No.1) in which the liver would be considered insufficient on clinical grounds, the heart cases were all well compensated, and in one of them the addition of a chronic interstitial nephritis made the excretion of salicylate the more unexpected. Hay's test was positive in only 25%, bilirubin was present in none of them, and urobilin was present in 25%.

In the cases that gave a negative result there was only one (No.14) in which a positive might have been anticipated. Hay's test was positive in one case and urobilin in one case.

The effect of anaesthesia upon the test was almost negligible, in only two cases was a positive salicylate test obtained, and in one of them it had been faintly positive before operation; in three cases the presence of bile salts in the urine was discovered after operation, but in one of them Hay's test had been positive before the operation.

It is not possible to form any definite conclusions as to the value of the test from so small a series - the results of the operated cases would suggest that the test was not so delicate as that of Widal, and the positive results in apparently normal cases would make the acceptance of the test difficult.

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PAPERS NOT REVIEWED.

- (1) Brodin. La. Medicine. 1: 625: July 1920.
  - (2) Loeper. Progres Med. 35: 205: 8th May, 1920.
  - (3) Garcia Guijarro. Sligo. Medica. Madrid. 68:  
124: 5th February, 1921.
  - (4) Einhorn. J.A.M.A. 81: 1494: Nov. 1923.
  - (5) De Martini. Riforme Medica, Naples. 40: 77:  
January, 1924.
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CONCLUSIONS.

It is not within the realms of practical possibility to test each of the numerous functions of the liver; it is necessary to select a few of the more easily assessable and to infer from them the manner in which the organ as a whole is performing its work. In the present state of knowledge it is permissible to argue in this way - from the particular to the general - but as the methods of testing the individual functions become perfected instances of isolated functional failure may be discovered. The matter is not, however, one of ~~the~~ very great moment as indubitable proof of our ability to test any single function is yet to be supplied.

The tests may be classified as (1) qualitative tests, (2) quantitative tests, and (3) tests of acute necrosis of the liver cells. It will be of some interest to pursue this classification a little further and to tabulate those tests which are of proved value in their respective categories.

(1) Qualitative Tests.

- (a) Hay's test.
- (b) Urobilinuria.
- (c) Bilirubinuria.
- (d) The glycuronic acid test.

(2) Quantitative Tests.

- (a) The phenoltetrachlorphthalein test.
- (b) The levulose tolerance test.

(3) Tests depending upon acute liver necrosis.

- (a) Leucine and tyrosine in the urine.
- (b) The Abderhalden reaction.
- (c) Blood lipase.
- (d) Blood and plasma fibrin.

When the criticism that has been made of each of these tests is recalled the prospect becomes rather depressing; each of the quantitative test has definite limitations - Rosenthal's test is suspected to be inapplicable in cases of biliary obstruction and the use of the levulose tolerance test depends upon the ability of the patient to retain the sugar.

Of the qualitative tests the glycuronic acid test is of value in prognosis as the absence of spontaneous and provoked glycuronuria usually heralds a fatal issue; the remaining tests in this group are strictly qualitative, and may be positive in even mild degrees of liver insufficiency.

Of the tests in the third group the elaborate technique needful in the estimation of blood and plasma fibrin, and in the performance of the Abderhalden reaction precludes their use in the clinique. The estimation of the lipase content of the serum may yet prove of value in prognosis, and the presence of leucine and tyrosine in the urine, is, of course, always sought in cases of suspected acute liver atrophy.

For/

For the testing of liver function (where acute necrosis of the hepatic cells is not sought) we are left with the following tests:-

- (1) The phenoltetrachlorophthalein test.
- (2) The levulose tolerance test.
- (3) Hay's Test.
- (4) The glycuronic acid test.
- (5) Urobilinuria.
- (6) Bilirubinuria.

Each of these tests is of definite value, but the levulose tolerance test would have to be omitted from routine work because of the objections that have been discussed in relation to its use.

In the routine examination of cases of liver disease or of suspected liver disease the five remaining tests will prove to have a definite use, at any rate they are the best known at the present time.

It is difficult to assume the role of a prophet, but in the future one would suggest that progress might be made along two lines.

- (1) The perfection of a method of estimating some constant constituent of the blood which is dependent for its concentration upon the normal functioning of the liver; this might be called the static test of liver function, and it would be comparable to the blood/

blood urea level in renal function testing. The writer has in mind the bile salt content of the serum.

(2) The discovery of some dye test which has not the obvious disadvantages of Rosenthal's method; the dye should be non-irritant, and capable of administration by subcutaneous or intramuscular injection, biliary permeability should have no influence upon its retention in the blood. This might be termed the dynamic test of liver function.

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